Biological control of *Acacia cyclops* in South Africa: the role of an introduced seed-feeding weevil, *Melanterius servulus* (Coleoptera: Curculionidae), together with indigenous seed-sucking bugs and birds.

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ABSTRACT

Acacia cyclops A. Cunn ex G. Don, or rooikrans (Fabaceae), of Australian origin, is an invasive environmental weed in South Africa where it threatens the unique vegetation of the Cape Floral Kingdom. The invasiveness of the plant in South Africa is, in part, due to its high annual seed-yields, together with the suitable climatic and edaphic factors found here. This study investigated the role of a seed-feeding weevil, *Melanterius servulus*, which was first introduced into South Africa during 1991, as a biological control agent of *A. cyclops* seeds, together with the combined effects of indigenous seed-sucking bugs and birds. The weevils utilise filled green pods of *A. cyclops* for adult feeding, oviposition and larval development and completely destroy seeds during these activities.

Examination of the reproductive phenology of *A. cyclops* showed that seeds are produced annually, during summer and that the presence of filled green pods on the plants coincides with the period of reproductive activity of the weevils. *Melanterius servulus* populations have established readily at all the original release sites, and within four years of release, damage levels to seeds commonly reached 90%. The weevils have dispersed at an average rate of approximately 2 km per year.

Since becoming established in South Africa, the seeds of *A. cyclops* have been utilised by a variety of generalist vertebrate and invertebrate organisms. Indigenous polyphagous alydid bugs feed on the mature seeds, rendering a portion of the crop inviable when damage levels are high (more than five feeding-punctures per seed). Several bird species are attracted to and feed on the fleshy aril that surrounds the seeds of *A. cyclops*. Passage of the seeds through the gut of birds enhances germination rates. There is a synergetic relationship between alydids and birds because seeds with low levels of alydid feeding (one to four punctures per seed) germinated more readily after passage through birds than seeds that were not eaten by birds.

A major part of this study examined interactions between *M. servulus* and the alydid bugs, because they indirectly compete for the same resource (seeds) with the weevils getting first use (immature seeds). The study showed that generally low levels of

damage to *A. cyclops* seed caused by native insects, together with increased germination levels in seeds that have been fed on by birds, justified the need for *M. servulus* in the system. The extensive levels of damage caused by the weevils have had no noticeable adverse effects on the bugs and the prospects are promising that the seed-feeding weevil will fulfil a substantial role as a biological control agent of *A. cyclops* in South Africa.

INTRODUCTION

The natural ecosystems of South Africa have become invaded by a variety of alien organisms, representing most of the major taxonomic groups (Henderson *et al.*, 1987; Macdonald *et al.*, 1986). Alien plants alone account for more than 8750 of the species that have been recorded in South Africa (van Wilgen *et al.*, 2002). An unusually high proportion of the plant species that have become invasive are trees and shrubs and these growth forms are the most prominent and damaging weeds (Richardson *et al.*, 1997). Most of the invasive trees and shrubs originate from regions of the world where climatic and edaphic factors are similar to those in South Africa, such as the Mediterranean Basin, California and Australia (Cowling and Richardson, 1995). These invasive alien plants are having substantial economic and ecological impacts, and managing such plants in South Africa, where large tracts of land are set aside for conservation, and where water is a limited resource, is a seemingly insurmountable task (van Wilgen *et al.*, 1998).

Several Australian *Acacia* species, and the closely related *Paraserianthes lophantha* (Willd.) Nielsen, have become pernicious weeds in agricultural lands, conservation areas, water courses and catchments in South Africa, and are also problematic elsewhere in Africa and in California, Chile and parts of Europe (Tutin *et al.*, 1968; Anon, 1977; Smith, 1979; Milton, 1980). Most of these species have the ability to alter nutrient cycling in the nutrient-poor environments they invade, by fixing atmospheric nitrogen, and, in South Africa, they have altered the landscape in regions of the country that were previously devoid of trees (van Wilgen *et al.*, 2002). Other problems created by invasive alien acacias are: increased fire hazards through the formation of dense stands of plants; reduction in crop and livestock production; and suppression of indigenous vegetation causing a loss in biodiversity (Macdonald and Richardson, 1986).

The proliferation of Australian *Acacia* species in South Africa, has been attributed mainly to the production of vast quantities of relatively long-lived seeds (Milton, 1980) because the plants are comparatively free from the insect and pathogen

destruction that occurs in their native range in Australia (van den Berg, 1977, 1980a, b, c, 1982a, b, c). The sizeable seed-banks that have been recorded in South Africa for these species (Milton and Hall, 1981; Holmes *et al.*, 1987b), are not achieved in Australia (Gill and Neser, 1984). Besides extensive campaigns to remove the invasive acacias using mechanical and chemical control methods, much of the control effort against these species has relied on the use of biological control agents, a process that has led to there being an unusually high number of biological control projects against alien tree species in South Africa, with 17 different species of trees targeted to date (Olckers and Hill, 1999; Zimmermann *et al.*, 2004).

The first agent to be released against an *Acacia* species in South Africa was a gall wasp *Trichilogaster acaciaelongifoliae* (on *A. longifolia* in 1982) that exacted very high levels of damage on its host plant (Dennill, 1985, 1987, 1988, 1990; Dennill and Donnelly, 1991). This success was followed by the introduction of *Trichilogaster signiventris* (Girault) onto *A. pycnantha* in 1987 that, after a slow start, resulted in equally impressive damage on the target weed (Hoffmann *et al.*, 2002). At about the same time, from 1985 onwards, seed-feeding weevils in the genus *Melanterius* were utilised against two other invasive *Acacia* species namely, *A. longifolia* and *A. melanoxylon*, as well as a species in the closely related genus, *Paraserianthes lophantha* (Dennill *et al.*, 1999). The ease with which the weevils became established and the obvious substantial levels of damage they caused on the seed crops of their host plants, encouraged the introduction of additional *Melanterius* species onto other *Acacia* species, including *A. cyclops* (commonly known as rooikrans).

In common with some of the other Australian *Acacia* species, *A. cyclops* was deliberately introduced into South Africa for its useful properties, in this case to protect urban dwellings and roads from shifting sand dunes (Shaughnessy, 1980). *Acacia cyclops* thrives in sandy, coastal habitats and after extensive propagation in these regions has become one of the most important invaders in the coastal fynbos biome (Macdonald and Jarman, 1984). The plants are now also utilised as a valuable source of fuel wood and so retain a useful property while simultaneously being a major problem (Azorin, 1992; Dennill *et al.*, 1999; Impson *et al.*, 2000). This double-edged-sword status has curbed prospects for biological control of *A. cyclops*, and other *Acacia* species, and accounts for the extensive use in South Africa of agents that

limit seed production but do not harm the vegetative growth of the plants. While the prospects are limited that plant density can be reduced, by limiting seed-production of these perennial trees, it is anticipated that rates of invasion will decline and overall management of the weed will be easier (Hoffmann *et al.*, 2004; Impson and Moran, 2004; Moran *et al.*, 2004; van Klinken *et al.*, 2004).

Although some information is documented on the effects of *Melanterius ventralis* on *A. longifolia* (Dennill and Donnelly, 1991; Donnelly, 1992, 1995; Donnelly and Hoffmann, 2004), published information on the role and effectiveness of the *Melanterius* species used so far as biocontrol agents, is scarce. The focus of this study is on the efficacy of the seed-feeding weevils, *Melanterius servulus* Pascoe, which are being used as biological control agents against *A. cyclops* in South Africa.

As a starting point it was important to trace the history of, and learn from, previous precedents in biological control programmes against the various Australian *Acacia* species. These issues are dealt with in Chapter 1, which also highlights the reasons why there were limitations on the choice of acceptable types of biocontrol agents, and why seed-reducing agents were selected as a first option.

It also became clear early on that it would be necessary to carry out a study on the reproductive phenology of the host-plant, *A. cyclops*, to investigate how closely this is linked to the reproductive biology of *M. servulus* (Chapter 2). In order for any seed-reducing agent to be effective it is essential that the timing of the production of 'utilisable' seeds by the host-plant, must coincide with the period of reproductive maturity and oviposition of the agent. Some reports had suggested that *A. cyclops* may produce seeds 'aseasonally', a scenario that would enable seeds to 'escape' beetle damage and thus lessen the overall control effort.

In Chapter 3 a framework is provided on which the assessment of the seed-feeding biocontrol agent is based. The chapter investigates in particular, the impacts of *M. servulus* adult feeding and larval damage on the canopy held seed of *A. cyclops*, whilst also quantifying the effects of adult feeding on the buds and immature pods of the plant. These effects were determined through monthly and annual evaluation of levels of damage by *M. servulus* to reproductive tissue of *A. cyclops*. The ability of a

biocontrol agent to disperse readily from its point of release is also a significant factor contributing to the success of that agent, and thus a preliminary investigation into the dispersal of *M. servulus* is also included in this chapter.

Monitoring the impact of *M. servulus* on *A. cyclops* presented some unusual challenges because the seeds of this species are also utilised by several species of invertebrates and vertebrates. Several bird species, in particular red-winged starlings (*Onychognathus morio*), are attracted to and feed on the fleshy red aril that surrounds the seeds (Glyphis *et al.*, 1981; Holmes *et al.*, 1987a; Holmes and Rebelo, 1988). In doing so they ingest the seeds, which then pass through the gut unharmed. This is one of the recognised dispersal mechanisms for *A. cyclops* seeds. A preliminary study had demonstrated that germination is enhanced in seeds that have passed through birds' digestive systems (Glyphis *et al.*, 1981). Feeding on *A. cyclops* seeds by indigenous seed-sucking bugs (Homoptera: Alydidae) also affects germination, sometimes adversely but otherwise favourably depending on the intensity of feeding. In addition, various rodents, ants, and a tortricid moth have been recorded to destroy seeds (David, 1980; Holmes, 1989; Donnelly and Stewart, 1990). To explore and discuss all these factors is beyond the scope of this thesis, but some aspects were considered further.

Whilst gaining an understanding of the impact of *M. servulus* on the canopy-held seeds of *A. cyclops*, it became impossible to ignore the potential impacts of birds and alydid bugs in the system. Despite the fact that there have been independent studies on the effects of bird and alydid feeding on the germination of *A. cyclops* seeds (Glyphis *et al.*, 1981; Holmes *et al.*, 1987a), no studies had investigated the two effects in combination. In Chapter 4, a laboratory study is described to determine the implications of bird and alydid feeding, both together and independently, on the germinability and viability of *A. cyclops* seeds.

The interaction between the alydid bugs and *M. servulus* was then singled out for further investigation (Chapter 5) because the weevils would be getting first use of the food resource (immature seeds) and thus potentially depriving the alydids of their food source (ripe seeds). The outcome of this exchange could effect the biological control programme either adversely (a scarcity of seeds causing the alydid populations

to crash) or favourably (damage levels on surviving seeds increasing due to a concentration effect of the same number of alydids feeding on fewer seeds).

Finally, the role of seed-feeders, and in particular the contribution of *M. servulus*, to the biological control of *A. cyclops*, is discussed.

CHAPTER 1

ACACIA CYCLOPS AND *MELANTERIUS SERVULUS* IN SOUTH AFRICA

1.1. Introduction of A. cyclops into South Africa

Acacia cyclops, is a shrubby, leguminous tree, belonging to the subgenus *Heterophyllum*. The plant is native to Australia where it grows in the climatically Mediterranean-type regions, extending from south-western Western Australia into South Australia (Figure 1.1, Maslin and Pedley, 1982). It was possibly first introduced into South Africa as early as 1835, as one of a number of *Acacia* species for Baron von Ludwig's public garden in Cape Town (Roux, 1961; Stirton, 1978; Shaughnessy, 1980).

Figure 1.1. Distribution of *Acacia cyclops* in Australia (after Maslin and Pedley, 1982).

Acacia cyclops, together with several other alien trees, became widely used from the mid 1850s onwards when concerted efforts were made to try and solve the problem of drift sands on roads in the sandy Cape Flats area and along the coastal regions of the

Western Cape Province (Shaughnessy, 1980). This problem had escalated over the years as a result of destruction of native vegetation through settlement and construction of roads, in addition to removal of plants for thatching and firewood (Shaughnessy, 1980). The climate, soils and fire regimes in the areas where *A. cyclops* was introduced in South Africa were comparable to those in its native land, allowing the plant to become one of the most important and widespread alien invaders of coastal areas (Macdonald and Jarman, 1984). It has now destroyed large areas of lowland fynbos in the Western and Eastern Cape Provinces (Figure 1.2), and continues to increasingly damage these sensitive ecological areas (Macdonald and Jarman, 1984; Boucher and Marais, 1995; Henderson, 2001).

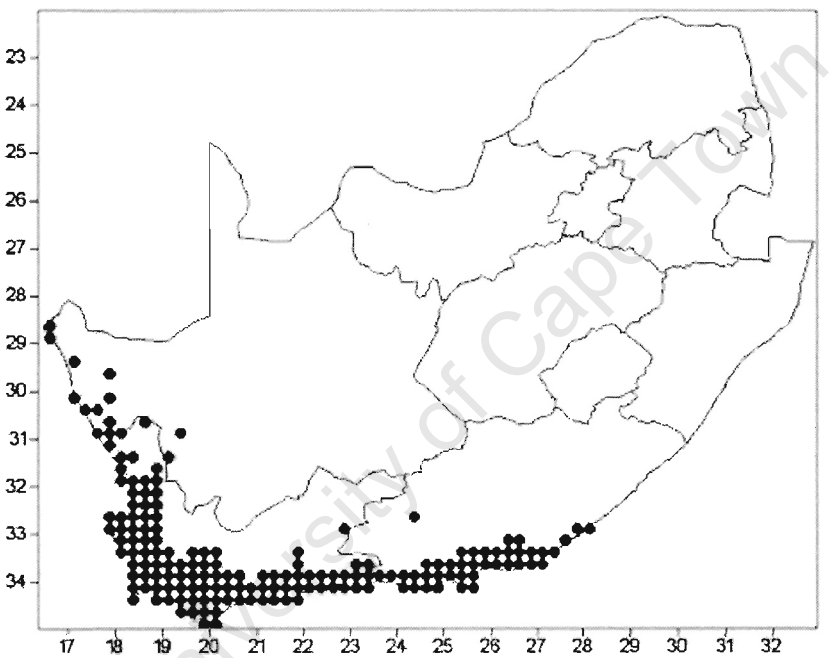


Figure 1.2. Distribution of *Acacia cyclops* in South Africa (after Henderson, 1995).

The fynbos biome, although very small (less than 90 000 km^2), is extremely rich in plant species diversity with some 8,600 species of flowering plants and ferns, and high levels of endemicity (Cowling and Richardson, 1995). Its uniqueness has made it one of the world's six Floral Kingdoms and in constant need of active conservation measures. Despite concerted efforts, the extent of pristine fynbos is declining, with the rapid spread of alien weeds, including *A. cyclops*, hastening the process.

1.2. Spread of Australian acacias in South Africa

Australian acacias are particularly successful invaders in the fynbos, and in a ranking of 33 invasive vascular plant species in this biome, seven of the top 15 weeds listed belonged to this genus, with *A. cyclops* ranked third on the list (Macdonald and Jarman, 1984). A number of factors have contributed to the success of these plants as invasive species in South Africa, the most important of which is probably their prolific seed production and large persistant seed-banks (Milton, 1980; Dean *et al.*, 1986; Holmes *et al.*, 1987b).

For the most part, Australian acacias are recorded as producing far larger seed crops in South Africa than in their native habitats (Weiss and Milton, 1984). For example, annual *A. longifolia* seed crops in the south-western Cape reach in the region of 2500 to 3500 seeds m^{-2}, as opposed to similar stands in Australia which yield only 300 to 400 seeds m^{-2} (Weiss and Milton, 1984). Annual seed production by *A. cyclops*, of 1000 to 5000 seeds m^{-2} (Milton and Hall, 1981; Holmes *et al.*, 1987b), is similar in the Cape and Australia, however the differences in the ratio of seed produced to seed stored helps to explain the invasive nature of alien acacias in South Africa (Dean *et al.*, 1986). In Australia five to seven *A. longifolia* seeds m^{-2} were measured in the seed bank, compared to 6000 to 9000 seeds m^{-2} in South Africa. The ratios of seed produced to seed stored were 60 and 0.4 respectively (Weiss and Milton, 1984). Ratios measured in Australia for *A. cyclops* were 23 and 53 (Gill and Neser, 1984), compared to 1,0 in South Africa, (Milton and Hall, 1981). In South Africa however, *A. cyclops* does not have the additional stress from herbivores and pathogens (Milton, 1980; van den Berg 1980a, b, c), even though an indigenous unidentified tortricid moth (Donnelly and Stewart, 1990) and at least two species of alydid bug (Holmes *et al.*, 1987a) have been reported to feed on the seeds. The amount of soil-stored seed in South Africa is much higher as a result of this.

Acacia cyclops seeds are also pre-adapted for bird dispersal, in that each shiny dark brown seed is surrounded by a large fleshy red aril, and seeds are retained and 'displayed' in open pods on the trees for a number of months (Turcek, 1963; Glyphis *et al.*, 1981; Knight, 1986; O'Dowd and Gill, 1986). Although many of the seeds are passively dispersed and fall just below the canopy, bird-assisted dispersal allows for

rapid and widespread movement of seeds into new, and often uninvaded, areas (Middlemiss, 1963). Additional factors contributing to the success of *A. cyclops* in South Africa are: its rapid growth rate, giving it the ability to reach reproductive maturity within about three years; good climatic matching between its native Australian range and areas it has infested in South Africa; and its pre-adaptation to nutrient poor soils, typical of the coastal and lowland areas that it has invaded.

1.3. Control mechanisms for A. cyclops

The necessity to control invasive Australian *Acacia* species in South Africa has been recognised for many years and considerable effort has been put into mechanical, chemical and biological control (Hall and Boucher, 1977; Dennill *et al.*, 1999). Furthermore, invasive alien trees and shrubs are believed to use approximately 7% (3300 million m^3) of South Africa's mean annual water runoff, and without any form of control this figure is likely to double within 15 years (Versveld *et al.*, 1998). In 1995 with the initiation of a national "Working for Water Programme" in South Africa, centrally-funded job opportunities for unskilled labour in impoverished communities were created, and large numbers of people are currently employed to remove alien plants from sensitive areas (van Wilgen *et al.*, 1998; Olckers, 1999, 2000; Kasrils, 2002). By clearing alien vegetation the Working for Water Programme not only aims to enhance water supply, but also to stabilize the ecology of natural ecosystems, and to restore the productivity of the land, whilst creating jobs and providing training (Gillespie *et al.*, 2004).

Despite the fact that above-ground infestations of these weeds can be removed effectively by integrating mechanical and chemical control, such methods are expensive and labour intensive, and regeneration of seedlings from the large seed-banks remains a constant source of re-invasion, requiring follow-up clearing operations. Biological control is thus seen as being an important component in the Working for Water programme, and has been identified as the only long-term self-sustaining method of managing the weed.

1.4. History of the biological control programme against A. cyclops

Early research on the biological control of *Acacia* species began almost 30 years ago with extensive surveys for natural enemies in Australia (van den Berg, 1973, 1977, 1980a, b, c). At the same time however the economic importance of several *Acacia* species meant that there could not be a *carte blanche* over the selection of potential agents (Impson and Moran, 2004). Australian acacias are considered by many to be economically beneficial, with *A. cyclops* and *A. saligna* supporting a R30 million charcoal and firewood industry in the greater Cape Town region alone (Azorin, 1992; McNeely, 2000). *Acacia mearnsii* and *A. melanoxylon* in particular, are commercially important in terms of tannin, paper pulp and timber production, and the conflicts of interest between those wanting to control the plant and those against control, placed constraints on the type of biocontrol agents that could be considered (Dennill and Donnelly, 1991).

Seed-attacking agents were proposed as preferred agents, due to their historical high degree of specificity (Janzen, 1975; Annecke, 1978; Auld, 1983), and the fact that they can curb the reproductive capacity of the target plants whilst not affecting their useful attributes. The proposal to use biological control against invasive *Acacia* species met with strong public objection (Stubbings, 1977), and protracted negotiations over 'acceptable' levels of seed damage were needed to eventually resolve the issue. By 1982 a bud-galling wasp, *Trichilogaster acaciaelongifoliae* had been released against *A. longifolia*, followed in 1985 by a seed-feeding weevil, *Melanterius ventralis* on the same target host. These introductions were followed by the release of *Melanterius acaciae* against *A. melanoxylon* in 1986.

Concerns about biological control of acacias resurfaced in 1987 with the pending release of a seed-feeding weevil, *M. servulus*, for the biological control of *P. lophantha*, a close relative of the Australian acacias. Tests in quarantine had shown that this weevil was able to feed, oviposit and develop within seeds of *A. mearnsii* (Donnelly, 1992), and despite the agreements of earlier negotiations, the release was opposed and the programme temporarily suspended. Part of the problem had arisen due to the development of a piece of agricultural legislation, known as the Wattle Act of 1987. The act allowed for various different associations and unions to

be represented on a growers' association that was formed to regulate the cultivation and production of *A. mearnsii* in South Africa. This Board was a separate body from the Wattle Research Institute that had earlier consented to the use of biological control against certain *Acacia* species (van Wilgen and McKenzie, 1987). A compromise was eventually reached with the Board in 1989 after it had been demonstrated that *A. mearnsii* seeds could be chemically protected from these weevils (Donnelly *et al.*, 1992), and *M. servulus* was subsequently released. Following this, in 1991, the biological control programme against *A. cyclops* began, with the introduction of yet another *Melanterius* species, referred to as *M. servulus* "Type A" (Dennill *et al.*, 1999). *Melanterius maculatus* was subsequently introduced for control of *A. mearnsii* in 1993, and more recently (2001) for *Acacia dealbata* and *Acacia decurrens*, while *Melanterius compactus* has been introduced against *A. saligna* (also 2001).

1.5. Taxonomy of the genus Melanterius

Melanterius species belong to the Curculionidae in the subfamily Cryptorhynchinae and tribe Ithyporini. The subtribe to which the genus belongs is essentially an Australian-Oriental group, with the greatest radiation of the genus having taken place in Australia (Donnelly, 1992). They have restricted host ranges, and the adults and larvae feed almost exclusively on the ripening seeds of acacias. Although some 88 species within the genus have been described (E.C. Zimmerman and R. Oberprieler, C.S.I.R.O. Canberra, personal communication, 2002), it was believed that the genus includes more discrete taxa than originally assumed (Dennill *et al.*, 1999). In particular there was some concern regarding the status of *M. servulus* and *M. maculatus*. Although both species were believed to be valid, different host records (*M. servulus* ex *A. cyclops* and *P. lophantha*, and *M. maculatus* ex *A. mearnsii*, *A. dealbata*, *A. decurrens* and *A. baileyana*) and slight behavioural differences implied that sub-specific strains or biotypes could be involved. A recent taxonomic review (Oberprieler and Zimmerman, 2001) and a molecular study (Clarke, 2002), of the species relevant to biocontrol of Australian *Acacia* species in South Africa could find no intraspecific differences within these groups and their nomenclature stands.

1.6. Biology of M. servulus

Melanterius servulus weevils are small (3-5mm), and red/brown in colour (Plate 1). They are univoltine, with breeding commencing in early Spring (September), which corresponds with the onset of pod maturation in *A. cyclops*. Adults feed on the green developing seeds, a process which appears to be necessary for maturation of the gonads (unpublished observations), and which renders the seeds inviable. Mating and oviposition follow, and the female weevils chew small holes through the walls of the swollen green pods, through which they insert a single egg. The eggs are deposited close to, or on, the seed surface, and the oviposition hole is then sealed. The newly hatched larvae burrow into the seeds where they feed and complete their development in approximately four to six weeks.

Plate 1. *Melanterius servulus* adult.

Generally only one larva develops per seed during which time it devours the entire contents of the seed, leaving only the hard seed coat. Unlike some other *Melanterius* species (*i.e.*, *M. maculatus*, *M. compactus*, and *M. ventralis*) the larvae do not feed on more than one seed. The fully developed larvae chew their way out of the seed and surrounding pod, and drop to the ground whereupon they burrow down (approximately 5-10 cm), and pupate within fragile chambers of compacted soil. Some larvae remain in the soil until the following breeding season, but for the most

part, fully developed adult weevils emerge from the soil some six to eight weeks later, where they remain mostly inactive for the cooler months, sheltering on their host plant or in the vicinity, usually in cracks and crevices under bark. These adults occasionally emerge to feed on the buds, flowers or new vegetative growth of *A. cyclops*, but are never seen in any great number until the following spring when the pods begin to enlarge.

1.7. Host-specificity studies

Prior to the release of *M. servulus* on *P. lophantha*, specificity studies were carried out, and release for this species had been approved. The specificity tests were conducted on a number of legume species, however due to the nature of the *Melanterius* life-cycle, and its requirements for green pods, testing in a quarantine room was not always feasible, and no-choice host-testing in outside sleeve cages was necessary (Donnelly, 1992). Test plants included some indigenous legume species, including several African acacias, several commercial legumes, and also some Australian *Acacia* species. Testing of African *Acacia* species was limited due to the flowering and fruiting periods, which were predominantly out of phase with the periods of *Melanterius* activity. Results of the tests showed *M. servulus* to be restricted to the *P. lophantha*/Australian acacia group, even though the host-range within this group was broad, and feeding, oviposition and larval development occurred on *P. lophantha* as well as the nine Australian *Acacia* species tested. The potentially wider host range of *M. servulus*, exhibited in these starvation tests was regarded as being a bonus, and since no commercial crops or indigenous legumes were under any threat, approval was granted for the release of the species (Donnelly, 1992).

1.8. Collections and releases of M. servulus

The first collection of *M. servulus* adults from *A. cyclops* for release in South Africa was made in October 1991 from Yanchep in Western Australia. These adults were released at Yzerfontein (33°20S 18°10E) in November the same year, and although some of these original beetles were later believed to have survived (in 1998), they reportedly failed to establish, and it was thought that the release had been made too

late in the season for the insects, and the pods had been too mature and unsuitable for oviposition.

In 1994, a further collection of some 3000 *M. servulus* adults was made in Esperance, Western Australia. These adults were released at 14 localities in the southwestern part of the Western Cape Province (Figure 1.3), including; (1) Baardskeerdersbos (34°34.511'S 19°28.120'E), (2, 3 & 4) three sites around Bredasdorp (34°31.650'S 20°02.012'E, 34°30.555'S 20°04.064'E, 34°33.119'S 20°03.030'E), (5) De Hoop Nature Reserve (34°24.117'S 20°32.867'E), (6) Gansbaai (34°44.237'S 19°37.730'E), (7) Gordons Bay (34°09.354'S 18°52.089'E), (8 & 9) two sites at Hangklip (34°22.706'S 18°49.822'E), (10) Hawston (34°24.143'S 19°07.493'E), (11) Napier (34°28.323'S 19°52.92'E), (12) Stanford (32°27.306'S 19°26.878'E), (13) Struisbaai/Springfield Estates (34°45.042'S 19°58.789'E) and (14) Vogelvlei near Pearly Beach (34°44.668'S 19°39.444'E).

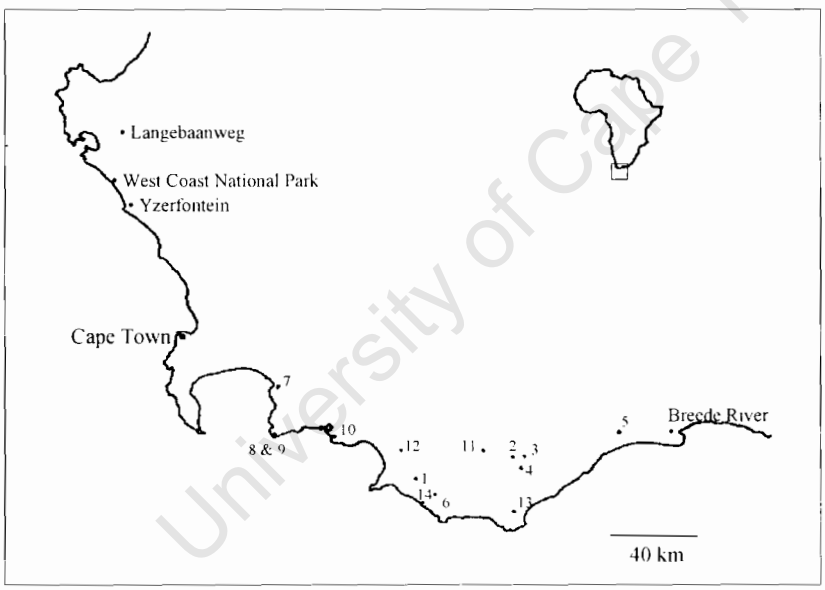


Figure 1.3. Map of the south-western part of the Western Cape Province showing the original *Melanterius servulus* release sites on *Acacia cyclops*. Numbers refer to those listed in the text for site names and grid co-ordinates.

Several of these sites have now become the focal points for collecting *M. servulus* adults for redistribution elsewhere in the country, and are also the areas in which much of this research was carried out.

CHAPTER 2

THE REPRODUCTIVE PHENOLOGY OF *ACACIA CYCLOPS*

INTRODUCTION

Dennill (1987) demonstrated the importance of host plant phenology studies when assessing the efficacy of biocontrol agents that effect the reproductive capacity of the target weed. He showed that following the introduction of *T. acaciaelongifoliae*, a gall-forming wasp which destroys the flower buds of *A. longifolia*, into South Africa, pod production of the host plant became cyclical with years of high pod production alternating with years of low pod production, during which vegetative growth was prolific (Dennill, 1987). The alternating cycle, together with the ability of the plant to produce higher pod loads where there is no water stress, reduced the overall impact of *T. acaciaelongifoliae* and necessitated the introduction of an additional agent, the seed-feeding weevil, *M. ventralis* (Dennill *et al.*, 1999; Donnelly and Hoffmann, 2004).

Milton and Moll (1982) recorded the vegetative and reproductive phenology of a number of Australian *Acacia* species with the purpose of providing information on which to base effective chemical, mechanical and biological control measures. Their study showed that for *A. cyclops* there were long periods during which ripe seed-pods were on the trees (from Spring through to early Autumn). Other unpublished reports (D. Donnelly, Plant Protection Research Institute, Stellenbosch) also mention that the production of ripe pods on *A. cyclops* occurs intermittently throughout the year. Neither of these reports provide the necessary quantitative information on the phenology of *A. cyclops*, so the patterns of flowering and pod production were unknown.

Of concern was the fact that aseasonal pod production could impact negatively on *M. servulus*. If the period of beetle activity does not coincide with pod ripening, some seeds will not be exposed to the beetles, with obvious implications for successful biological control of the weed species. Any such deficiency could be compounded if

there are inter-year fluctuations in pod production of the plants, as has been found in *A. longifolia* (Dennill, 1987). As a result, surveys were conducted to determine whether or not there are asynchronies in the long-term phenology of *A. cyclops* that might reduce the biological control effectiveness of *M. servulus*.

METHODS

Six sites were selected in the Western Cape Province to monitor the reproductive phenology of *A. cyclops*. Three of these sites were on the eastern coastal plain, near the mouth of the Breede river (34°24.443 S 20°49.546), at De Hoop Nature Reserve (Potberg) and near Bredasdorp (34°30.555 S 20°04.064 E), and the other three were on the western coastal plain at Yzerfontein (33°20 S 18°10 E), the West Coast National Park (33°16.097 S 18°10.054 E) and the West coast Fossil Park at Langebaanweg (33°58.062 S 18°05.549 E), (see Figure 1.3, Chapter 1, for relative positioning of these sites). The first three sites were monitored for a two-year period from March 1999 to March 2001 (to ascertain if there were indeed differences in the reproductive phenology of the plant from one year to the next), and the last three were monitored for a single year between August 1999 and September 2000.

Reproductive phenology was measured in a non-destructive manner. All the *A. cyclops* trees that were monitored were growing as a monoculture, were reproductively mature, and part of a dense infestation where canopy cover was complete. In these situations, clumps of several trees aggregate to form what is effectively a single very large tree with many stems. The collective canopy of these aggregations forms a single outer layer where growth of the plants takes place. Beneath the canopy is a shaded region where very little plant growth occurs. Only actively growing branches positioned on the perimeter of the canopy were monitored.

At each of the six sites, ten branches bearing reproductive material, all on separate trees, were haphazardly selected at a reachable height and each branch was tagged at a point 70 cm from the tip. The growth form of *A. cyclops* is such that reproductive tissue is produced towards the branch tips each year, so the 70 cm length accounted for all the reproductive tissue on the branch, and all new growth would also be

accounted for during the study period. Each site was visited at monthly intervals when counts were made of all the living reproductive components on all of the tagged branches. The reproductive components were differentiated into flower buds, open flowers, immature pods, unfilled green pods, filled green pods, ripe pods and old pods. The different pod categories were distinguished as follows: 'immature pods' represented the initial podlets which develop just after flowering, that were cylindrical, short and stubby, 'unfilled green' included pods that were elongated, but the seeds within them were still small and undeveloped; 'filled green' included pods that were swollen with almost fully-developed seeds; 'ripe' included pods that had turned brown and contained hardened seeds but had not split open; 'old' pods had dehisced and started to shed their seed.

At the Breede river site, during the first sampling year, reproductive productivity was relatively low, and it became clear during the second year of sampling that the ten tagged branches were not truly representative of trees at this site. For this reason a further five branches were selected and monitored for the duration of the second year in addition to the original ten branches.

Data analysis

Main effects Analysis of Variance (Statistica 7.0), using site and year as the fixed factors, was used to compare temporal changes in the reproductive capacity of *A. cyclops* trees by analyzing the counts of filled pods on plants on the east coast in the 1999/2000 and 2000/2001 growing seasons. One way ANOVA was also used to compare spatial differences in the reproductive capacity of *A. cyclops* trees by analyzing the counts of filled pods growing on the east coast as opposed to the west coast during the 1999/2000 growing season. Only the filled pods were used as a comparative measure for statistical analysis, since this is the main reproductive stage that is utilised by *M. servulus*. Comparisons were made for October, which was the month during which filled pods were most abundant. The phenology of the plants was compared visually by plotting the proportions of each component on the plants at different times of year.

In order to elucidate whether phenological differences might occur between *A. cyclops* trees on the eastern and western coastal plains, the representative sites from each coast were considered separately. The mean number of each of the reproductive components (± the standard error) was calculated for the month when the particular component was at its most abundant. The 'filled' green pod stage was specifically selected to demonstrate any temporal or spatial differences in productivity.

RESULTS AND DISCUSSION

On the eastern coastal plain, there were significant differences between the three sites in the numbers of filled green pods that were produced ($F_{(2,58)} = 6.14$, $P = 0.004$) (Table 1), although the differences between pod production in the first and second years were not significant ($F_{(1,58)} = 3.7$, $P = 0.06$). Owing to changes in the monitoring procedure at the Breede river site, this was then excluded from the analysis, with the result that no significant difference was apparent in pod production between the remaining sites (Bredasdorp and De Hoop) ($F_{(1,34)} = 0.91$, $P = 0.35$). There was however a difference in pod production between the two years ($F_{(1,34)} = 12.64$, $P = 0.001$), indicating evidence of alternation in reproductive potential between years. Since only one branch per tree was marked for the monitoring in this study, this alternation in reproductive potential is only indicated at the branch level, and is not necessarily a reflection of the status of the tree as a whole. Personal observations made during the course of this study suggest that *A. cyclops* branches show alternating reproductive and vegetative growth, but at any given time an *A. cyclops* tree will have both reproductive and vegetative branches present. Therefore, unlike *A. longifolia*, where reproductive growth is cyclical, this situation is not likely to be of concern as far as *M. servulus* populations are concerned. The reproductive capacity of plants growing on the eastern and western coastal planes, once again with the exception of the Breede river site, were not significantly different ($F_{(1,49)} = 1.602$, $P = 0.2$).

Table 1. Annual representation of mean monthly counts (± S.E.) of reproductive material on 10 branches of *Acacia cyclops* at each of three sites on the eastern coastal plain and three sites on the western coastal plain.

	Eastern coastal plain		**Western coastal plain**
	Year 1 (1999/2000)	**Year 2 (2000/01)**	**Year 1 (1999-2000)**
	Bredasdorp	**Bredasdorp**	**Yzerfontein**
# buds	48.2 ± 12.5	193.5 ± 64.2	66.3 ± 25.9
# flowers	1.7 ± 0.75		30.1 ± 19.1
# immature pods	236.9 ± 60.1	194.8 ± 67.1	139.5 ± 61.02
# unfilled green pods	9.1 ± 3.2	13.4 ± 7.6	8.1 ± 2.5
# filled green pods	33.6 ± 6.9	27 ± 7.02	38.4 ± 15.1
# ripe pods	55.3 ± 11.7		34.1 ± 11.2
# old pods	8.2 ± 3.8	46.5 ± 10.1	37.4 ± 12.8
	De Hoop Nat. Res.	**De Hoop Nat. Res.**	**Langebaanweg**
# buds	16.2 ± 12.6	8.5 ± 4.6	24 ± 8.5
# flowers	26.2 ± 8.02	0.1 ± 0.1	16.3 ± 7.8
# immature pods	356.4 ± 101.6	2.1 ± 1.2	158.2 ± 63.6
# unfilled green pods	44.8 ± 11.14	5.0 ± 1.3	17.5 ± 6.12
# filled green pods	41.6 ± 7.8	2.9 ± 0.86	52.2 ± 7.5
# ripe pods	52.9 ± 10.9	4.8 ± 1.4	56.1 ± 9.8
# old pods	9.2 ± 2.4	17.1 ± 6.9	54.4 ± 7.9
	Breede River	**Breede River**	**W/coast Nat. Park**
# buds	112.7 ± 40.6	66.4 ± 35.3	25.1 ± 11.04
# flowers	58.5 ± 13.7	52.2 ± 34.9	23.5 ± 11.97
# immature pods	26.7 ± 14.9	71.3 ± 41.2	139.5 ± 61.02
# unfilled green pods	0.1 ± 0.1	14.3 ± 4.4	17.9 ± 5.16
# filled green pods	0.5 ± 0.4	12.6 ± 4.01	58.9 ± 13.5
# ripe pods	3.9 ± 2.8	17.3 ± 4.8	48.0 ± 10.8
# old pods	38.8 ± 11.1	5.1 ± 2.4	45.5 ± 12.6

Although the information in Table 1 provides the means of all the reproductive plant parts produced during the year, it does not give any indication of the timing of reproductive activity or the proportional representation of each reproductive stage during the course of the year. Like many of the other Australian *Acacia* species, *A. cyclops* produces large numbers of flower buds and flowers each year. In most

cases approximately 50 % of the buds produced do not progress to develop into flowers, however this species is different to many in that it does not suffer major losses between the flowering stage and the production of immature pods, and flower abortion (except for extremely windy conditions), is minimal (personal observations). Such a reproductive strategy results in the production of numerous clusters of immature podlets. The greatest reproductive losses occur to the pods between the immature and unfilled green pod stage. Based on field observations it would appear that as some of the pods in the clusters begin to elongate, they also drain much of the energy resources, and in doing so do not allow for all podlets within the cluster to reach maturity and many of the little immature podlets eventually desiccate and die. Most of the unfilled green pods continue to develop and form fully-developed, filled, green pods, which in turn ripen and split open. Pod persistence is the norm in *A. cyclops*, accounting for the large numbers of very old pods on the trees. This feature has also been noted in several other Australian *Acacia* species (*i.e.*, *A. melanoxylon* and *A. implexa*), and is typical of those acacias bearing seeds on fleshy red or orange arils that are dispersed by fructivores (Pedley, 1978; Milton and Moll, 1982).

Figures 2.1 and 2.2 illustrate the timing of the appearance and disappearance of the reproductive components on the plants as well as the relative abundance of each component at different times of the year. The periods over which each stage was present were consistent between years and between sites. However, for the eastern coastal sites there were differences in the relative proportions of reproductive plant parts between the first and second years (e.g. there were proportionately more unfilled green pods during September 2000 than during September 1999) (Figure 2.1). The sites on the western coastal plain were slightly more advanced in the timing of the onset of each stage during this study period, but this may not be consistently so for every year.

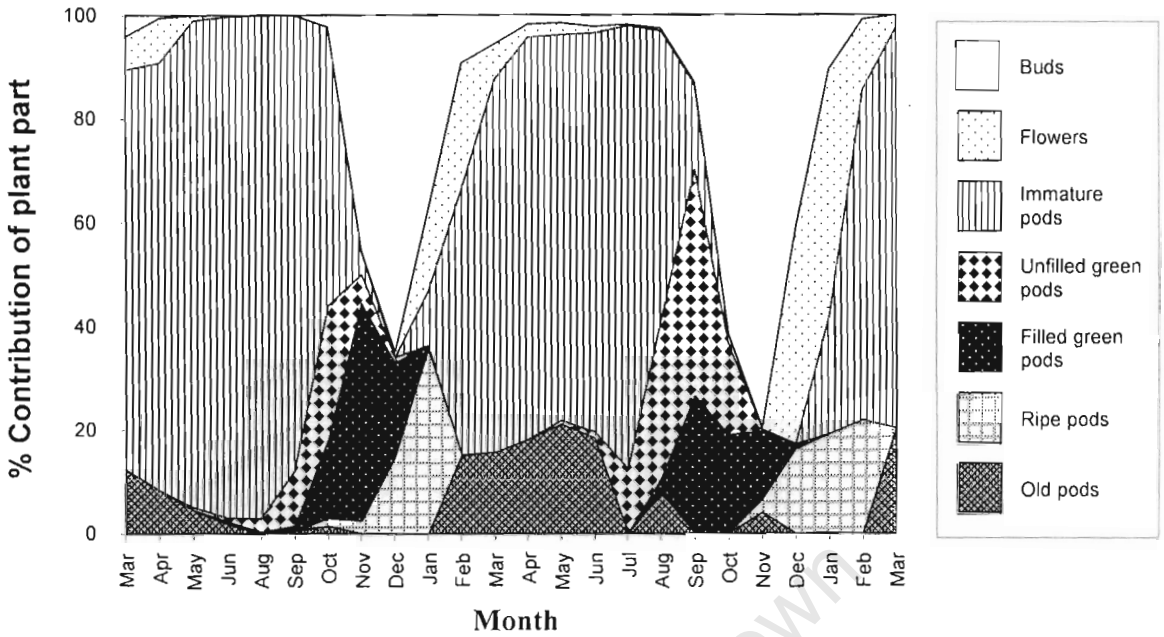


Figure 2.1. Reproductive phenology of *Acacia cyclops* on the eastern coastal plain, showing the proportion of the various reproductive plant parts over two years for all sites combined.

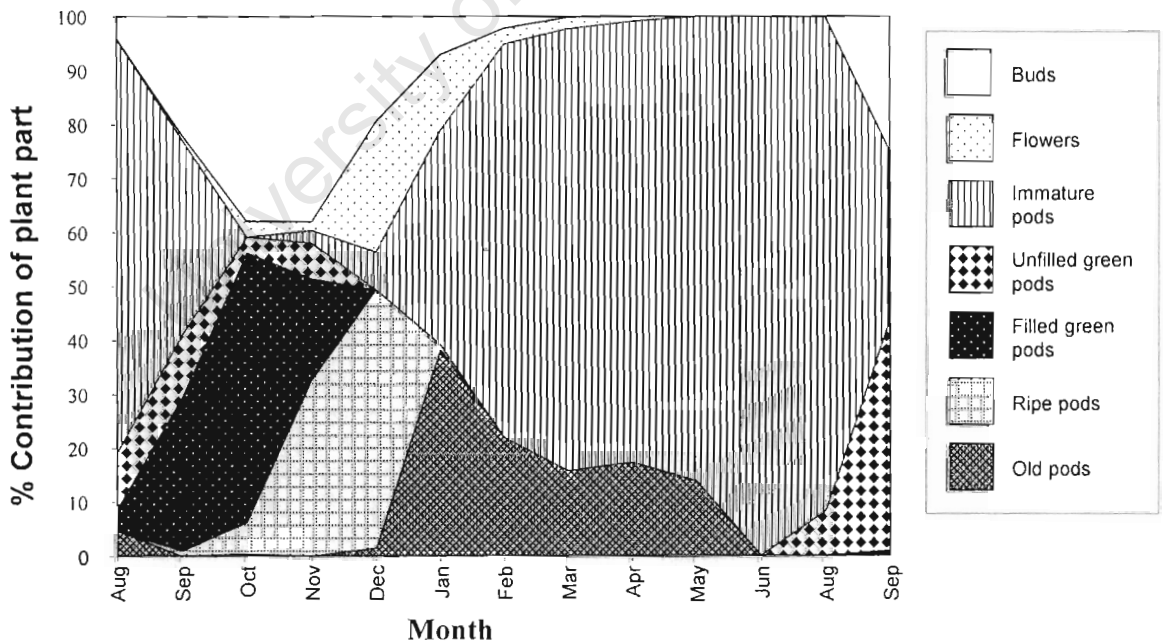


Figure 2.2. Reproductive phenology of *Acacia cyclops* on the western coastal plain, showing the proportion of various reproductive plant parts over one year for all sites combined.

Flower buds were most abundant during October/November, although they were present in low numbers on the plant throughout the rest of the year. Flowering peaked during December and January, however flowers were sometimes produced at other times, and occasional flowers were found on some plants throughout much of the year. Immature podlets developed from these flowers and the podlets were held in small clusters (of up to 40 pods), which slowly lengthened and matured into unfilled pods during the autumn and winter months.

The reproductive stage that is most utilised for feeding and oviposition by *M. servulus* is the filled green pod stage. On both the eastern and western coastal plains this stage is only present during the spring and summer months. Seed pods in this condition were present from June through to January during the 1999/2000 season, and from July to January in the 2000/2001 season. However the proportion of these pods on the trees was very small initially, reaching a peak in October and tailing off into January. The peak in October coincides directly with the period when ovipositing *M. servulus* females are most active (personal observations, see Chapter 3, Figure 3.3). There is a window period when the seeds might escape damage, between June, when filled pods first appear, and August/September, when *M. servulus* becomes active. However, filled pods were extremely scarce during this period (Figures 2.1 and 2.2). Besides which, these pods, and the seeds within them, only begin hardening into ripe pods during September, by which time the weevils have started feeding. As *M. servulus* is able to utilise the seeds until the pods have started to turn brown, pods that mature to the filled green stage early are not unavailable to the beetles and thus there are no potentially limiting asynchronies between the phenologies of the weevils and their host plant.

By studying the reproductive phenology of *A. cyclops*, not only has the synchrony between the host-plant and its biological control agent been demonstrated in this particular case, but phenological studies also provide important information for the planning and timing of alternate biological, chemical or mechanical control methods. Such studies have also given baseline data for *A. cyclops*, both pre-biocontrol (Milton and Moll, 1982) and with the addition of a single seed-reducing agent, and will allow future research to monitor the impacts that any additional biological control agents may have on the reproductive output of the plant.

CHAPTER 3

ABUNDANCE AND DISPERSAL OF THE SEED-FEEDING WEEVIL, *MELANTERIUS SERVULUS*

INTRODUCTION

For many years, the potential for the utilization of seed-reducing agents in biological control programmes has been debated. Many biocontrol practitioners have felt that such agents are unlikely to succeed, because they are required to be consistently, highly damaging to have any impact, and even in such situations they do not necessarily reduce populations of the weed (Harris, 1973; Kriticos *et al.*, 1999; Myers and Risley, 2000). There is continuing controversy over seed-limitation and propagule pressure as regulators of plant populations (Crawley, 1989, 1990, 1992; Louda, 1989; Turnbull *et al.*, 2000; McEvoy, 2002). In situations where recruitment is not seed limited, seed-reducing insects are unlikely to have a measurable impact on the plant populations (Crawley, 1989). Julien (1992) lists most of the examples of insects that reduce the reproductive potential of plants as failures. Despite this, seed-reducing agents have been frequently used and are widely accepted, particularly to suppress the invasiveness (*i.e.*, rates of spread and densification) of woody weeds, and have shown that they can make a substantial contribution to controlling the target weed when levels of seed destruction are high (Goeden, 1978; Kluge, 1983, 1989; Harley, 1985; Cloutier and Watson, 1990; Kluge and Neser, 1991; Julien, 1992; Hoffmann and Moran, 1998). There are other reasons for the selection and use of seed-reducing agents in biological control of weeds, notably the high degree of specificity within the guild (Janzen, 1971, 1975; Annecke, 1978); and the chance to minimize potential conflicts of interest, (De Loach, 1981; Neser and Moran, 1985; Dennill and Donnelly, 1991; Impson and Moran, 2004).

Seeds are foci for new invasions, and the higher the number of seeds produced, the greater the threat of invasion into new areas (Harper, 1977; Neser and Kluger, 1986; Moody and Mack, 1988; Rejmánek, 1996; Williamson and Fitter, 1996; Rouget and Richardson, 2003). Tree taxa that generally display the most rapid rates of spread are

those that mature early, have high reproductive capacity and long-distance seed dispersal (Clark, 1998; Caswell *et al.*, 2003), all traits of several of the invasive Australian acacias in South Africa. Consequently, by decreasing the number of seeds being produced by a plant, the rate of spread and colonization of new areas will be slowed through a reduction in the amount of seed falling into safe and or un-colonized sites, and by reducing the distance from the source at which population recruitment occurs (Janzen, 1970; Harper, 1977; Macdonald and Jarman, 1984; Foster, 2001). The reduction of seed may also have an important role to play in the overall management of invasive plants, by substantially lowering efforts and cost of follow-up clearing treatments after initial clearing has taken place (Moran *et al.*, 2004).

Seed-feeding weevils, all in the genus *Melanterius*, have been selected and utilised for the biological control programme against various Australian *Acacia* species in South Africa since 1985 (Dennill and Donnelly, 1991; Dennill *et al.*, 1999; Impson and Moran, 2004). These *Melanterius* weevils show a high degree of specificity to the Australian plants in the genus *Acacia* and the closely related *Paraserianthes lophantha* (van den Berg, 1980b, 1982c; Auld, 1983; New, 1983), and do not attack any of the African acacias or related species (Donnelly, 1992). Within the genus *Melanterius* however, the degree of host specificity is less clear. Although some species are only ever associated with a single acacia host, others feed as adults on several acacia hosts (New, 1979, 1983; Auld, 1983; Donnelly, 1992). For example, *M. servulus*, occurs on both *A. cyclops* and *P. lophantha* in the field in Australia and South Africa (See Chapter 1; van den Berg, 1980b; Oberprieller and Zimmermann, 2001), and during host-specificity tests in cages was found to feed, oviposit and develop on seeds of eight other Australian *Acacia* species (Donnelly, 1992).

Subsequent to the first release of *M. servulus* against *A. cyclops* in 1991, efforts were renewed in 1994 to collect and make further releases of the weevil in South Africa (Dennill *et al.*, 1999; Impson and Moran, 2004). Experience with the early programmes against *A. longifolia*, *A. melanoxylon* and *P. lophantha*, had shown that the weevils were sufficiently host specific, relatively easy to collect, and readily available (Impson and Moran, 2004). The use of *M. servulus* against the seeds of *A. cyclops* in South Africa also had the added benefit of not being opposed by groups that rely on the plants as a source of income, as would have happened had other types

of more-destructive agents been selected. *Acacia cyclops* is widely used in this country as a source of excellent fire wood, and it is harvested in many of the areas where it occurs not only for own use, but also to generate income, particularly among the poorer communities (Azorin, 1992). By using a seed-feeding weevil, steps have been taken towards reducing the aggressiveness and spread of *A. cyclops* without hampering its beneficial qualities.

Eight years after the main releases in 1994, *M. servulus* populations were monitored in an effort to understand the impacts that the weevils are having on seed output of their host. Although establishment of the beetles was confirmed at most release sites the year following release, initial levels of seed damage were based on visual estimates of pods *in situ*. No destructive sampling was carried out because there was concern that such methods might impact negatively on the newly-established, and small weevil populations (D. Donnelly, P.P.R.I., Stellenbosch, personal communication).

Donnellys' initial estimates of damage were not at all promising, with many of the release sites showing an estimated < 0.01% destruction of seeds by the weevils after three years, and a maximum damage level of approximately 5% which was recorded at only one site (D. Donnelly, unpublished data). In contrast, the two programmes that had been initiated several years earlier, using the seed-feeding weevils, *M. ventralis* and *M. acaciae* on *A. longifolia* and *A. melanoxylon* respectively, showed a steady increase in damage over time reaching 79.5% after only a few years (Dennill and Donnelly, 1991; Donnelly, 1995). These two programmes had also shown that the insects were relatively slow to disperse away from the release sites into adjoining infestations of the weeds, indicating that *M. servulus* would also not spread rapidly. The *Melanterius* weevils are winged, so disperse by flying from one area to the next, although dispersal events may also be assisted by prevailing winds. The initial impact assessments of *M. servulus*, coupled with its probable limited dispersal, cast doubts on the ability of *M. servulus* ever becoming an effective biocontrol agent of *A. cyclops*.

This study was undertaken to monitor *M. servulus* more thoroughly by using destructive sampling methods to assess both the impact of adult feeding on flower

buds and immature seeds, as well as adult feeding and larval development on mature seeds at, and at varying distances from, the original release sites.

METHODS

Impact of M. servulus on immature reproductive growth of A. cyclops

Six *A. cyclops* infestations in the Western Cape Province (South Africa) were selected as sample sites for the study. Three of the sites were on the western coastal plain at; Langebaanweg, Yzerfontein and the West Coast National Park (33°16.097 S 18°10.054 E), and the other three were on the eastern coastal plain; near the mouth of the Breede River (34°24.443 S 20°49.546 E), at De Hoop Nature Reserve (Potberg) and near Bredasdorp (34°30.555 S 20°04.064 E) (see Chapter 1, Figure 1.3). All of the sites, except for the West Coast National Park and the site near the Breede River mouth, had established populations of *M. servulus*, and were the same sites that were selected for the phenology studies described in Chapter 2. Shortly after the start of the sampling programme, a large portion of the Bredasdorp infestation was cleared and this site was discarded.

To determine the impact of *M. servulus* adults on the immature reproductive growth (flower buds and podlets) of *A. cyclops*, the terminal section of an indiscriminately-selected branch, approximately 50cm long, was removed from each of ten trees at monthly intervals for one year. This was carried out between September 1999 and September 2000 at each of the six study sites described above, ensuring that none of the tagged branches being used for the phenology study were tampered with. The West Coast National Park and the Breede River sites had no *M. servulus* and acted as controls. A small consignment of *M. servulus* was released close to the study site at the Breede River mouth in October 1999, and although the weevils had established by the following year, they did not disperse into the study area and thus did not influence observations or counts undertaken at this site.

Each month the 60 sample branches were dismantled, and a count was made of all the reproductive components on each branch. The flower buds, podlets, and pods were then examined microscopically for signs of *M. servulus* feeding damage.

Sweep samples were taken to determine when *M. servulus* adults were active in the field and thus potentially contributing to the feeding damage that was recorded on the various reproductive stages of the plant. These samples were taken at monthly intervals for one year (September 1999 to September 2000) at each of the six main sites. Each sweep sample entailed shaking a cluster of *A. cyclops* branches into a large insect net, to dislodge and capture any free-living stages of insects, from each of ten trees at each of the six sites. Once again, those branches being used for the phenology study were excluded from the sweep sampling to avoid possible damage to the plant parts. A record was made of the numbers of *M. servulus* and also any other phytophages caught in the net, and the reproductive phenology of the branches that were sampled was described (*i.e.*, the presence of buds, flowers, and/or pods was noted), to ascertain which part of the plant the phytophages were feeding on.

Impact of M. servulus on mature reproductive growth of A. cyclops

During late November to early December, when *A. cyclops* seeds had matured, and after *M. servulus* adults had fed and oviposited on the seeds and the resulting larvae had developed within them, a detailed examination of the seed pods was made to determine the levels of seed damage. This aspect of the study was carried out annually between 1998 and 2002 at 14 selected sites, for the most part where *M. servulus* had originally been released (including four of the sites – Langebaanweg, Yzerfontein, Bredasdorp and Potberg, that had been sampled for levels of damage on immature reproductive growth as described above). (see Table 3.2).

At each of the 14 sites, between 30 and 50 ripe pods (*i.e.*, filled pods that have just hardened) were collected indiscriminately from each of five trees. A sub-sample of ten pods was randomly selected (unsighted, from a paper bag) from each of the batches of pods, giving 50 pods in total per site. Each pod was opened and a record was made of: i) seeds damaged by adult feeding (Plates 2a, b and c); ii) seeds damaged by larvae (Plate 3); iii) seeds on which oviposition had occurred (Plate 4);

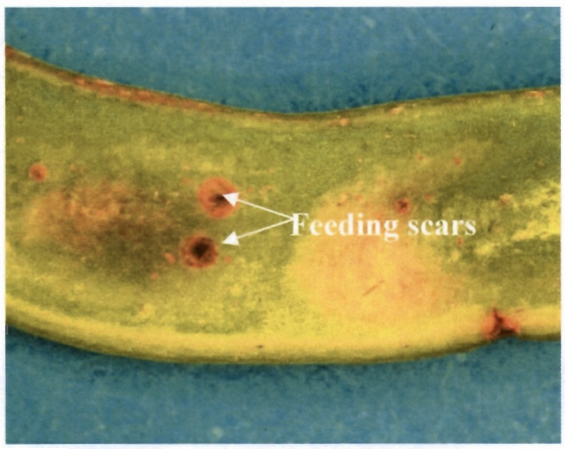


Plate 2a. *M. servulus* adult feeding damage on external surface of green ripening pod.

Plate 2b. Adult feeding damage as seen on external surface of ripe pod.

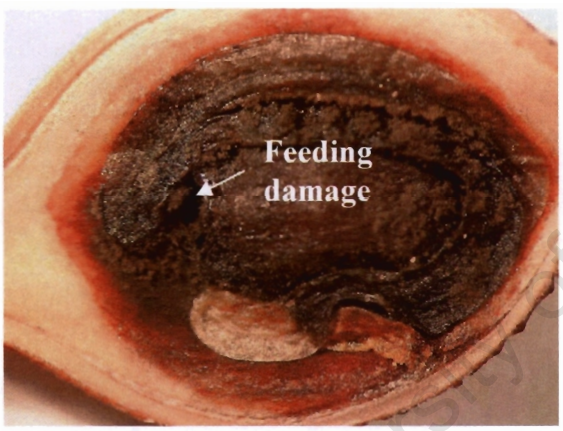


Plate 2c. Internal view of seed within pod to show effects of adult feeding.

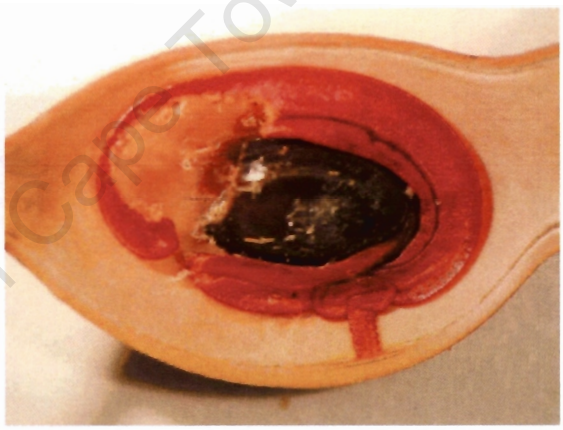


Plate 3. Internal view of seed within pod to show effects of larval feeding.

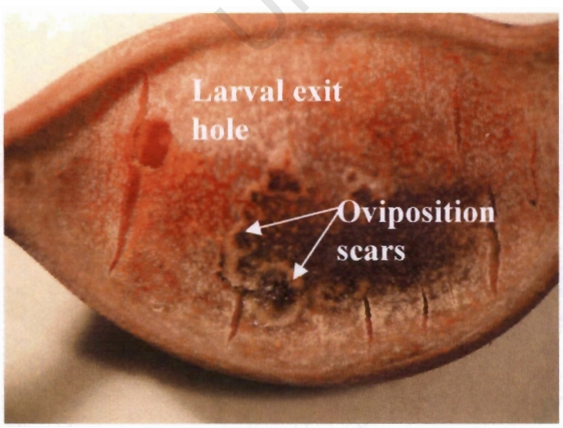


Plate 4. External surface of pod showing oviposition scars and the larval exit hole.

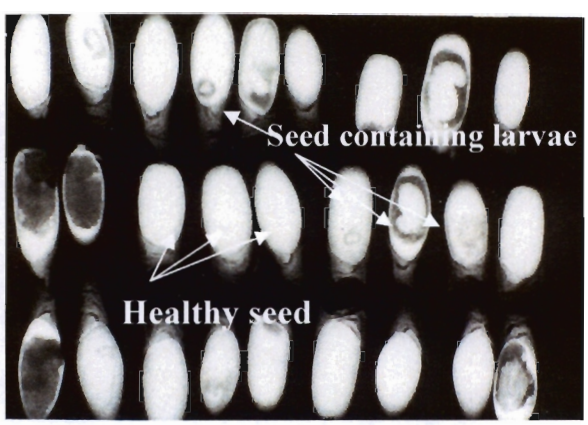


Plate 5. X-ray sheet showing healthy seed and seed colonised with larvae.

iv) non-viable, seeds (where other forms of damage not attributable to *M. servulus* damage had occurred); v) aborted seeds and vi) seemingly healthy seeds.

For the first year of the study, due to uncertainties in distinguishing oviposition damage to the pods, seeds in categories iii) and vi) were X-rayed. This was done by retaining the seeds between two sheets of transparent, adhesive paper and placing them on plates (Agfa Gevaert, 8 x 10" Osray-C film), within an X-ray machine (Softex Co. Ltd., Tokyo, Japan No. 6701) at 13 KVP and 9 mA for a duration of 60 seconds. The film was then developed in an automatic developing machine in the radiology unit at Stellenbosch Hospital. The developed film was examined on a light table, and any seeds containing *M. servulus* larvae were easily identified (See Plate 5). In the years subsequent to this, X-ray examination of the seeds was not necessary because with experience, oviposition scars on the outside and inside of the pod walls could be distinguished with confidence using a dissecting microscope.

Dispersal of M. servulus

Dispersal of *M. servulus* from seven of the original release sites was measured in December 1999 and again at selected sites (Langebaanweg and Yzerfontein) in December 2001. This was the optimal time to look for signs of dispersal of the weevil because, damage by the insects on *A. cyclops* pods was easily detectable (*i.e.*, presence of adult feeding and oviposition scars on the external surface of the pod walls and characteristic, round, holes through which the fully developed larvae had emerged prior to dropping to the soil to pupate). The study entailed inspection of pods on a number of *A. cyclops* plants at increasing distances (approximately every 500m) in a 14 km radius around the original release points. Where possible, collections were made radiating from as many compass directions as possible, however this was restricted to a large part by accessibility of roads and availability of trees. At each inspection point three people independently searched *A. cyclops* trees for signs of *M. servulus* damage. If no signs of damage were observed within ten minutes, *M. servulus* was assumed to be absent from the area, the search was discontinued and no pods were collected. Where damage was noted, approximately 25 seed-pods were indiscriminately collected from each of ten trees, and a sub-sample of ten pods per tree was then scrutinized to ascertain levels of seed damage. Changes in levels of damage with distances from the release site were

compared between 1999 and 2000 at two of the sites to determine whether there was an increase in damage levels and dispersal distances with time. The co-ordinates, for each locality that was surveyed for signs of damage, were recorded irrespective of whether damage was noted or not.

Data analysis

Feeding damage on immature reproductive growth caused by *M. servulus*, was calculated as the overall percentages of each component damaged by combining counts from each of the sample months and all trees at each site. The relative proportions of damage caused by adult feeding, oviposition and larval feeding were compared by Analysis of Covariance (Statistica 7.0), with years being the covariable, on arcsin transformed percentages of damage in each category at different levels of overall seed damage. Linear regression of $\log_{10}$ arcsin transformed percentage seed damage on $\log_{10}$ distance + 1 from release sites was used to show dispersal distances of *M. servulus* in South Africa. Analysis of Covariance, (using distance as the covariable), was used to compare changes in levels of percentage damage (arcsin transformed) at different distances from original release points in 1999 as opposed to 2001.

RESULTS

Impact of M. servulus on immature reproductive growth of A. cyclops

Only two indigenous phytophage taxa were recorded routinely together with *M. servulus* at all of the study sites in the sweep samples on *A. cyclops* (Figures 3.1, 3.2, 3.3). These included an unidentified pentatomid species, possibly *Antestiopsis orbitalis* (Hemiptera: Pentatomidae), which is known to have adapted to feeding on the immature seeds of some of the Australian *Acacia* species (Picker *et al.*, 2002). The other taxon consisted of at least two species of Alydidae, one of which had been previously recorded and identified as *Zulubius acaciaphagus*, (Schaffner, 1987), and both of which are well known polyphages feeding on the mature (hardened) seeds of both exotic and indigenous acacias (Holmes *et al.*, 1987a).

Figures 3.1 a-e show the periods during which time the pentatomids were present on the *A. cyclops* trees at the five sites. They were only collected in large numbers at Yzerfontein, being scarce at the other four sites. Although the pentatomids were present from spring to late summer, and even into autumn, they showed a preference for feeding on filled green pods (leaving signs of small pierce marks on the pod walls, personal observations). Pods in this stage were only available in abundance during October. The levels of damage caused by the pentatomids on *A. cyclops*, although not quantified, were never extensive (personal observations).

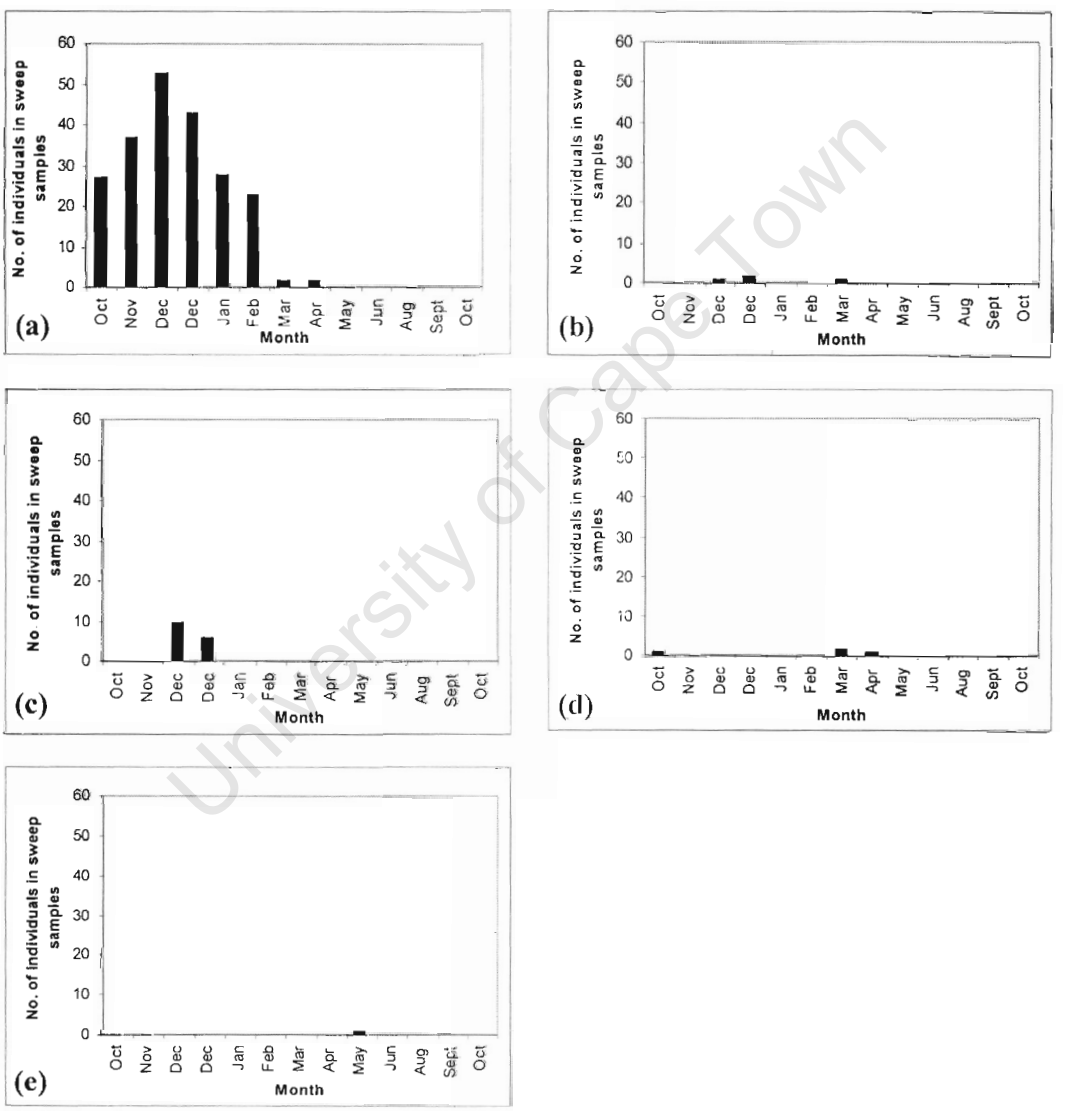


Figure 3.1. Abundance of pentatomid adults and nymphs on *Acacia cyclops*, at each of five study sites; (a) Yzerfontein, (b) Langebaanweg, (c) West Coast National Park, (d) Potberg, and (e) Breede River.

Alydids were generally more abundant than pentatomids at the study sites (Figures 3.2 a-e), with Yzerfontein (Figure 3.2a) again showing the highest levels of abundance. Alydids feed, for the most part, on the hardened seeds within ripe pods that have split open, which explains their peak in abundance in sweep samples from mid to late summer when ripe pods are prolific. The adults probably also feed on the filled green seeds, but levels of damage to these seeds were not ascertained. Damage to the ripe and hardened seeds by alydid feeding is dealt with in Chapter 4.

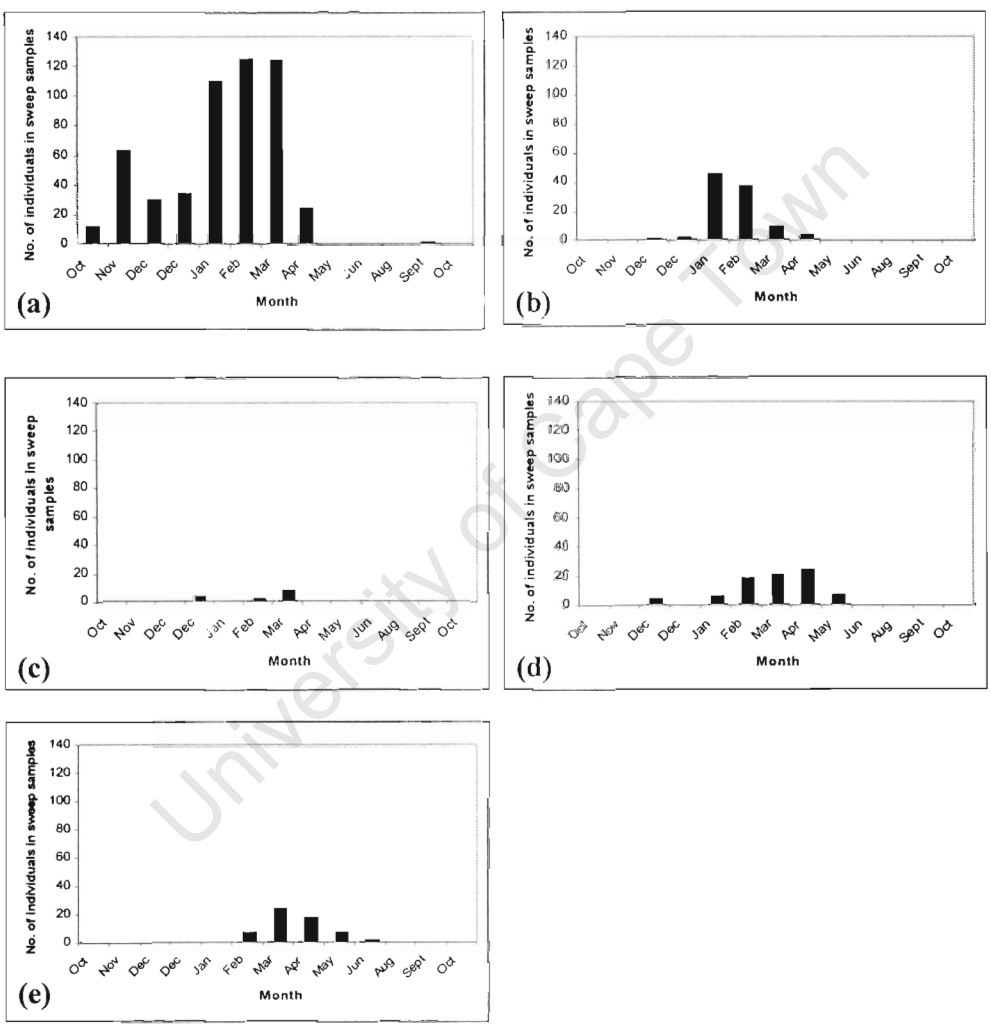


Figure 3.2. Abundance of alydid adults and nymphs on *Acacia cyclops* at each of five study sites; (a) Yzerfontein, (b) Langebaanweg, (c) West Coast National Park, (d) Potberg, and (e) Breede River.

Melanterius servulus adults, despite being the least abundant species in the sweep samples, are the most damaging of the phythophages present on *A. cyclops*. For much of the year they are inactive and not present on the terminal shoots, and are only abundant during the spring and summer months (Figures 3.3 a-c), when their predominant food source (green seeds within filled green pods) becomes available (see Chapter 2).

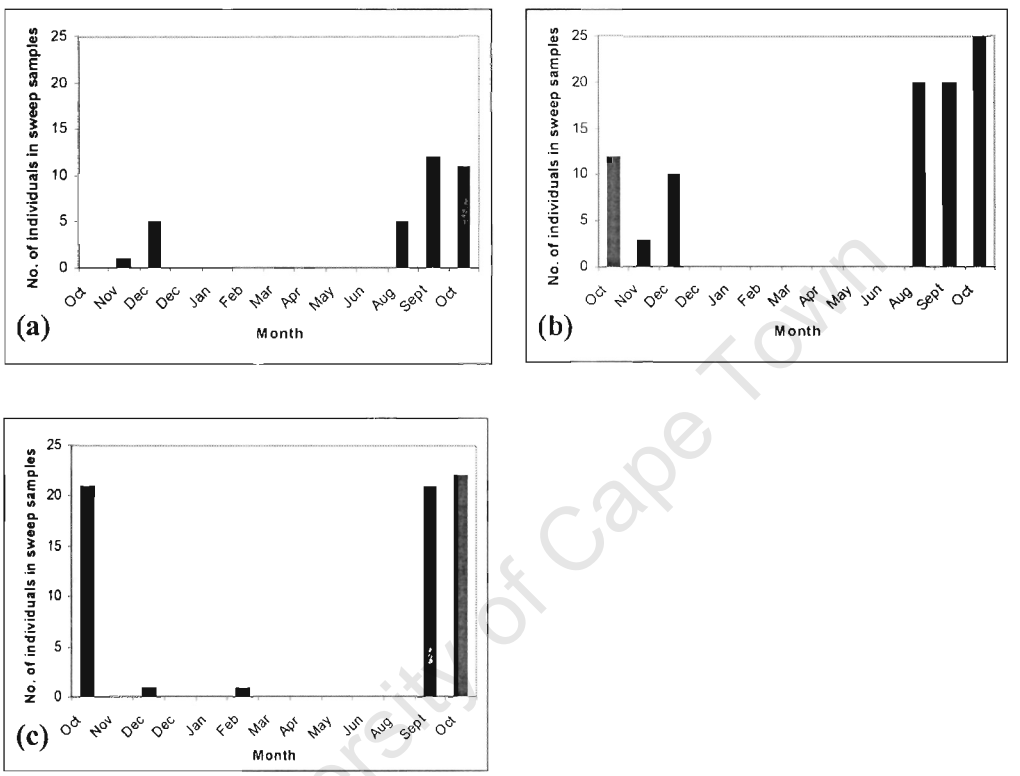


Figure 3.3. Abundance of *Melanterius servulus* adults on *Acacia cyclops*, monitored from sweep samples at three study sites; (a) Yzerfontein, (b) Langebaanweg and (c) Potberg.

Although there was only a single record from the sweep samples of one *M. servulus* adult being collected in Potberg out of the peak season, adults were observed occasionally wandering on the extremities of the branches, away from their usual over-wintering refugia under bark. On warm, sunny days between January and August, *M. servulus* adults become active and feed on any available parts of the *A. cyclops* plants (*i.e.*, buds, pollen from the flowers, podlets and young vegetative growth). No *M. servulus* individuals were recorded or seen from either the West Coast National Park

or the Breede river sites, confirming these two sites as weevil-free controls for the duration of the study.

Monthly destructive sampling of immature reproductive growth showed that some *M. servulus* feeding occurred on this stage of growth (Table 3.1). The feeding damage by the adult weevils was noticeable as small shot holes on the bud and podlet surfaces. This type of damage was however negligible and is unlikely to make a significant contribution to the productivity of the weed. By contrast the levels of damage attributable to *M. servulus* was much higher on filled pods (Table 3.1). Since no damage was recorded from either the West Coast National Park or the Breede River sites, where both pentatomids and alydids were present, the damage observed at the remaining sites is attributable to feeding by *M. servulus*, and not the other indigenous phytophages.

Table 3.1. Damage (%) caused by *Melanterius servulus* on the reproductive stages of *Acacia cyclops* during a one-year period (1999-2000), at five localities. (Numbers of each of the components examined at each site are shown in parentheses.)

	Locality				
Stage	Yzerfontein	Langebaanweg	West Coast Natnl Park	De Hoop, Potberg	Breede River
Flower buds	5.3 (187)	0.8 (124)	0 (125)	2.6 (303)	0 (69)
Immature pods	0.1 (5268)	0.8 (4631)	0 (4288)	3.1 (2455)	0 (3701)
Unfilled pods	1.6 (749)	6.5 (1077)	0 (759)	7.9 (926)	0 (971)
Filled pods	45.9 (50)	60.6 (50)	0 (50)	48.6 (50)	0 (50)

Impact of M. servulus on mature reproductive growth of A. cyclops

The damage that is inflicted by *M. servulus* adults and larvae on seeds within the filled green pods is of relevance to the biocontrol programme (Table 3.2). There was no significant difference in overall levels of *M. servulus* damage on *A. cyclops* seeds between years (Table 3.2) (Kruskal-Wallis $H_{(4, N=52)} = 1.858$; $P = 0.76$), however, there

were highly significant differences in levels of damage between sites (Kruskal-Wallace $H_{(14,\ N=52)} = 39.777$; $P = 0.0003$). These differences were not found to be attributable to the length of time the beetles had been established at each site (r = -0.21; ns, for damage levels in 1999).

Table 3.2. Overall seed damage (%) caused by *Melanterius servulus* on filled pods of *Acacia cyclops* over a five-year period at different release sites. (blank spaces represent sites not sampled during that year). The approximate number of weevils released at each of the sites is given in parentheses.

Release site	Release	Seed damage (%)				
	date (No.)	1998	1999	2000	2001	2002
Langebaanweg	1997 (1000+)		65.8	67.4	93.1	50.8
Yzerfontein	1991 (100)		47.5	58.8	61.0	70.0
Bredasdorp West	1994 (200)	26.3	14.9		28.6	29.1
Bredasdorp South	1994 (250)	0.0	14.8		13.2	2.1
Bredasdorp East	1994 (300)	70.5	10.8	23.4	63.5	64.8
Potberg	1994 (200)	74.5	47.8	86.6	94.4	
Springfield	1994 (200)		3.0			23.6
Struisbaai	1994 (250)	20.8	28.1		94.0	38.0
Gansbaai	1994 (300)		7.1		31.6	32.4
Hangklip	1994 (200)	80.8	97.1		70.1	
Baardkeerdersbos	1994 (200)	12.5	29.5		14.5	30.6
Hawston	1994 (200)	98.0	98.7		93.8	
Stanford	1994 (200)	96.1	82.1		79.0	87.3
Napier	1994 (200)	7.7	11.3			72.1

At some of the sites, damage levels fluctuated markedly between years (e.g., Struisbaai and Potberg), whilst others were either consistently high (e.g., Hawston, Stanford and

Hangklip), or consistently low (e.g., Bredasdorp South and West). It was notable that damage levels in excess of 90% were recorded at several sites at least in some years.

Damage to the ripe green seed by *M. servulus* (resulting in seed mortality), was categorized into three types: (i) adult feeding, which left a distinctive perforation through the pod wall (Plates 2a and b), with a cavity chewed into the underlying seed that rendered the seed inviable (Plate 2c); (ii) feeding damage caused by the larval stages, which consumed the entire contents of the seed, leaving only the hard exocarp intact (Plate 3); and (iii) oviposition probes caused a high proportion of seed mortality – this category was distinct because, although eggs may or may not have been deposited, the oviposition sequence was incomplete and the perforations had not been plugged as happened after normal oviposition events. The seeds below the oviposition probes were no longer shiny black and healthy, but poorly developed, and frequently shrivelled or mouldy, with an aril that was yellow, rather than red, in colour (see Plate 6).

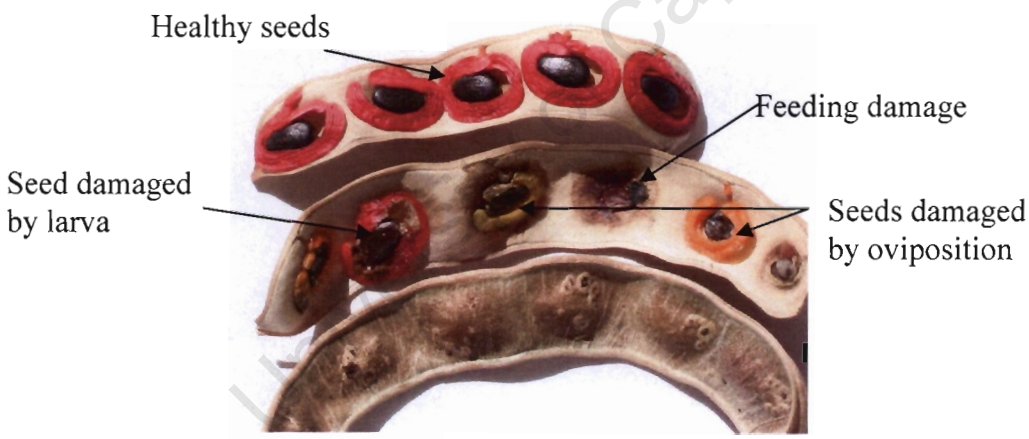


Plate 6. *Acacia cyclops* pods, showing pod with healthy seeds (top) and an opened pod with damaged seeds (middle). Oviposition and feeding scars can be seen on the outer pod wall (bottom).

At all of the sites where *M. servulus* damage was recorded, oviposition damage constituted the largest portion of overall damage to the seeds, and was more than three times greater than both larval damage and adult feeding damage (Figure 3.4).

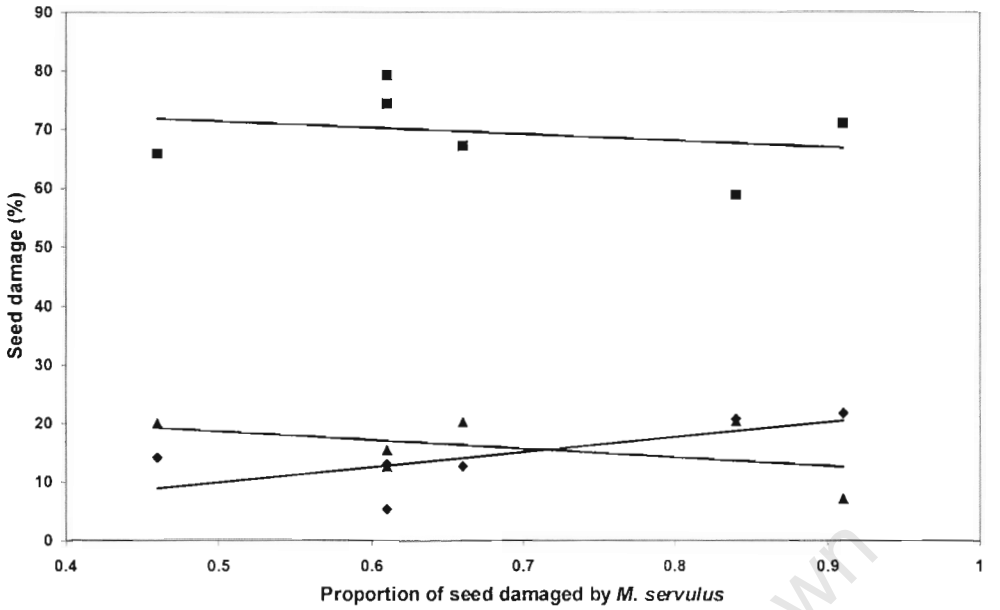


Figure 3.4. Percentage damage attributable to adult feeding (♦), oviposition (■) and larval damage (▲) by *Melanterius servulus* on *Acacia cyclops* at different levels of overall damage, as measured over a 3-year period at four sites combined.

An analysis of covariance showed that, regardless of overall levels of damage, there was no difference in levels of damage attributable to adult as opposed to larval feeding ($F_{(1,9)} = 2.12$; $P = 0.179$), but that oviposition damage was always significantly greater than adult and larval damage ($F_{(1,14)} = 22.66$; $P = 0.0003$)

Dispersal of *M. servulus*

No previous records of dispersal rates for *Melanterius* weevils existed, and this study, carried out between five to seven years post-release of the biocontrol agent, is the first to demonstrate the level of dispersal ability by these weevils. Dispersal was measured around seven of the release sites (Figures 3.5 a-g). The pattern shows that the highest populations of weevils, (as illustrated by high seed damage levels), have remained close to the release points. There was a significant decline ($R^2 = 0.35$, $P << 0.0001$) in the levels of seed damage recorded in 1999, with increasing distance away from the release points (Figure 3.5h).

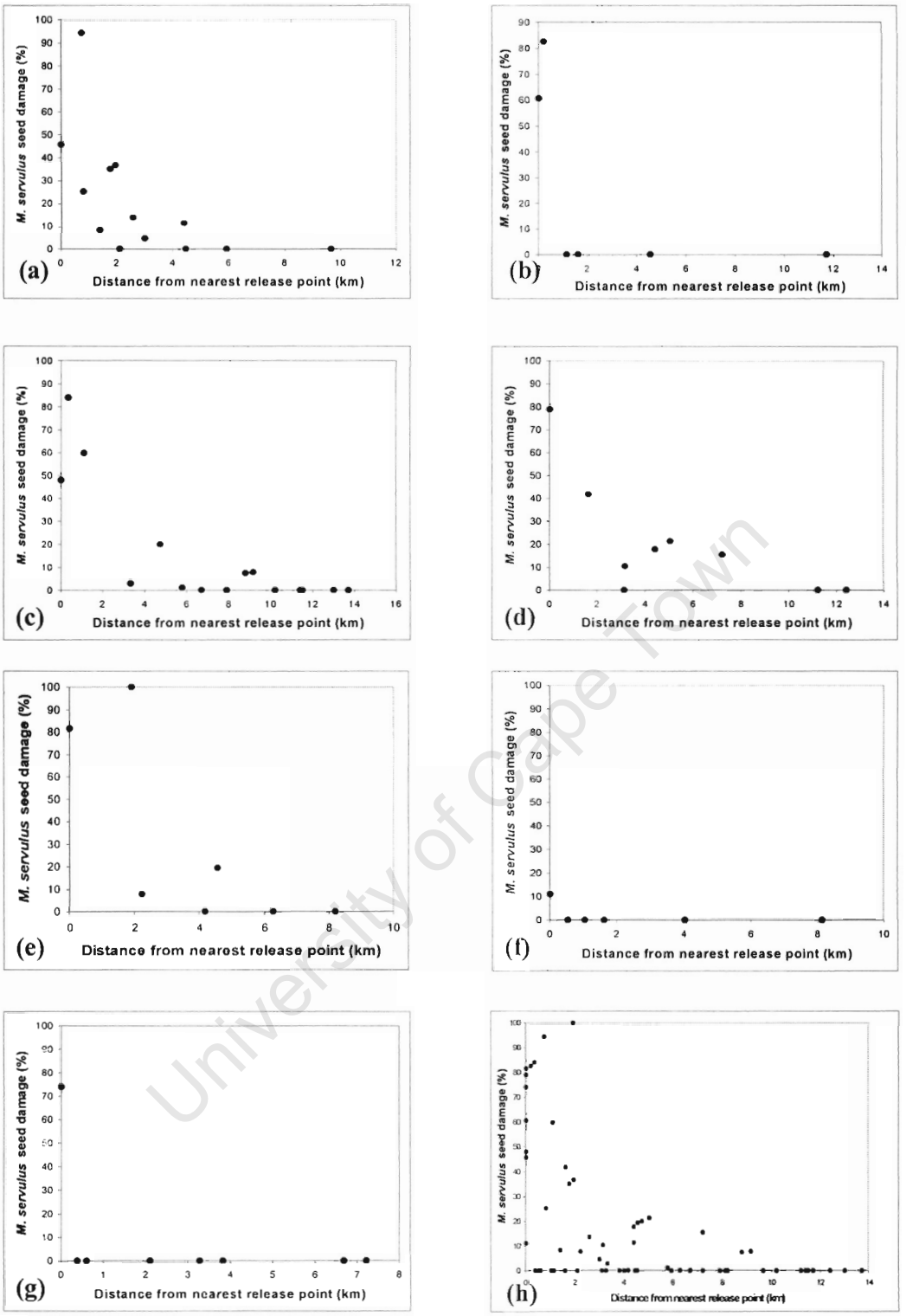


Figure 3.5. Dispersal of *Melanterius servulus* as shown by overall seed damage levels (%), in 1999, around seven release sites, (a) Yzerfontein, (b) Langebaanweg, (c) Potberg, (d) Stanford, (e) Hawston, (f) Bredasdorp, (g) Hangklip, five years after the release of the agent. (h) illustrates all sites combined.

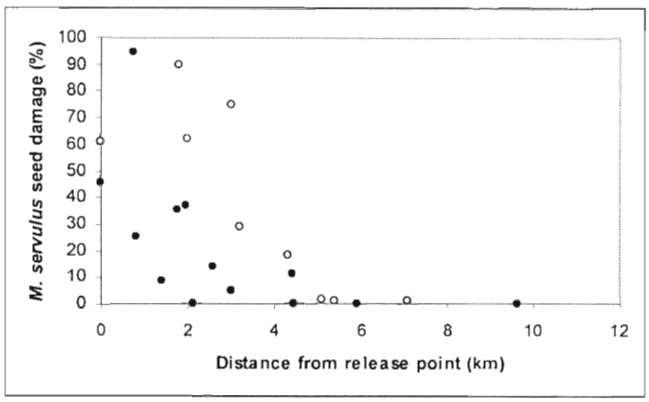


Figure 3.6. Expansion of range by *Melanterius servulus* from 1999 (closed circles) to 2001 (open circles) at Yzerfontein, showing seed damage levels (%) at each of the monitoring points.

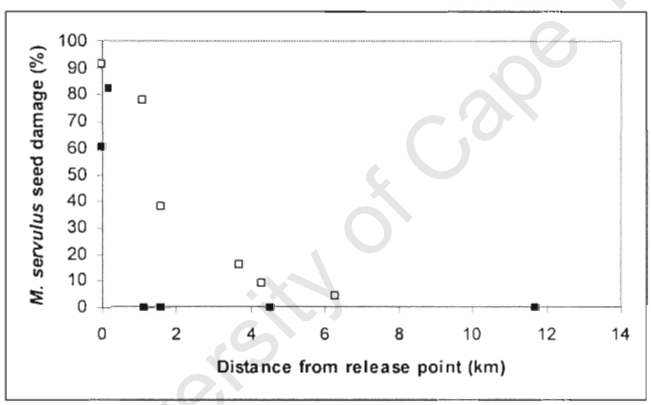


Figure 3.7. Expansion of range by *Melanterius servulus* from 1999 (closed squares) to 2001 (open squares) at Langebaanweg, showing seed damage levels (%) at each of the monitoring points.

Figures 3.6 and 3.7 show comparative levels of damage at Yzerfontein and Langebaanweg in 1999 and 2001. There was a highly significant decline in the levels of damage with increasing distance from the release sites ($F_{(1,29)} = 40.19$; $P << 0.00001$) in both years, reflecting the moderate rates of dispersal of the beetles. However there was a significant increase in levels of damage between 1999 and 2001 ($F_{(1,29)} = 10.37$; $P = 0.003$), showing that the beetle populations were expanding in the vicinity of the two release sites.

Melanterius servulus populations are probably still under a dilution effect as the populations expand spatially. If so, the seed damage levels recorded in Table 3.2 could increase and stabilize with time and currently might be an under-estimate of the true potential of the agent. In 1999, the furthest distance from any release site where damage was recorded was 9km. This was measured at Potberg (Figure 3.5c), five years after initial release of *M. servulus*, showing that the agent is able to disperse, on average, at least 1.8km per year. It is important to note however that this relationship is not linear, but dependent on the number of insects released into an area, as well as the size and density of the weed infestation, and rates of dispersal could well increase with time.

DISCUSSION

Much of the previous knowledge of Australian acacia seed mortality by *Melanterius* species is based on studies in Australia (van den Berg, 1977; Auld, 1983; and New, 1983), and more recently in South Africa (Dennill and Donnelly, 1991; Donnelly, 1992, 1995; Dennill *et al*., 1999; and Impson *et al*., 2000). More specifically, records of 25% and 20% seed mortality were reported for *A. cyclops* in Australia (van den Berg, 1977; New, 1983) and it was suggested that *Melanterius* populations were "rarely likely to become sufficiently dense to cause other than extremely sporadic competition for seeds" (New, 1983). These records are in complete contrast to the findings of Janzen (1975, 1980) on central American *Acacia* species, where high levels (more than 70%) of seed mortality by bruchid beetles were recorded. Unlike the Americas, Israel and Africa, where bruchids are the principal seed-feeders of *Acacia* species (Lamprey *et al*., 1974; Carr, 1976; Ross, 1979; Coe and Coe, 1987; Barnes, 2001), weevils in the genus *Melanterius* predominate on the Australian acacias (New, 1983). In contrast to the Bruchidae, *Melanterius* weevils are not susceptible to parasitism, however it is possible that low levels of fecundity, coupled with competition from other seed-feeders in their native country, could restrict their potential for being particularly destructive. However, the evaluation of seed mortality in Australia (van den Berg, 1977; New, 1983) did not clarify on what basis mortality was measured, and thus if oviposition damage was not taken into consideration, this alone could account for the differences recorded between Australia and South Africa.

This study of *M. servulus* on *A. cyclops*, together with other evaluations of *Melanterius* species on *A. longifolia* (Dennill and Donnelly, 1991), *A. melanoxylon* (Donnelly, 1995), *P. lophantha* (Schmidt *et al.*, 1999) and *A. mearnsii* (F. Impson, unpublished data) have provided insights into the potential of this particular species, and the genus *Melanterius* as a whole, as far as biological control efforts are concerned. Despite a poor prognosis from initial estimates for the impact by *M. servulus*, there has been a general trend towards increasing levels of seed-damage following release of the weevils into new areas. Dispersal of *M. servulus* away from these areas, although relatively slow, is taking place, and will probably increase with time as populations of the weevils build up. The efforts involved in the process, from selection of this weevil species in its country of origin, through to its release in South Africa, were thus well founded (Impson and Moran, 2004).

The current study has certainly not answered all the questions regarding the potential effectiveness of *M. servulus* as a biocontrol agent of *A. cyclops* seeds, but rather provided a framework on which to build additional information. Further investigation is required into inter- and intra-site differences in seed-damage levels. In particular, the role of changes in annual seed-production on the plants, extraneous disturbances of the sites (e.g., removal of trees for firewood, clearing operations, occurrence of fires, and dust from roads and roadsides), dispersal of the weevils and habitat characteristics (e.g., soil type, climate etc.) needs to be ascertained.

In order to evaluate the full potential of *M. servulus* as a biological control agent of *A. cyclops* it would be necessary to investigate whether the reduction in seed production, as measured, translates into a reduction in *A. cyclops* populations or at least a reduction in its rate of spread. Providing that seed-production is reduced and consistently maintained below a certain critical level, it could be expected that the plant populations would demonstrate a corresponding decline, particularly in the case of plants with relatively short-lived seed-banks (Hoffmann and Moran, 1998). Australian acacias in general are reputed to have very large and long-lived seed banks, and thus even with levels of 80-90% seed-damage, biocontrol of the seeds alone is likely to be ineffectual. Such arguments have been supported by models based on a study of seed-feeders on *A. nilotica* (L.) Delile (Kriticos *et al.*, 1999), and observations of a flower

bud-feeder on *Sesbania punicea* (Cav.) Benth., (Hoffmann and Moran, 1991). In the case of *A. cyclops*, there are still uncertainties about seed dynamics, and although it is believed that the seed-bank is probably relatively short-lived in comparison to some of the other invasive Australian acacias (*i.e.*, *A. mearnsii* and *A. saligna*), further studies are required on seed-banks, seedling recruitment and plant population dynamics to verify the potential role of seed-feeders in the system.

The actual value of seed-reducing agents in this, and other, systems may only be fully realized by determining what is meant and required by "effective" biological control (see the Synopsis to this thesis). Seed-reducing agents are likely to play an important role in terms of integrated weed management. In South Africa, where large-scale mechanical clearing operations are being undertaken, such as those by the 'Working for Water' programme, this strategy is believed to be playing a substantial role in the overall management of invasive plants. Where seeds of invasive species are at reduced levels, it is believed that there will be a corresponding decrease in costs and effort of clearing per unit area, particularly during follow-up treatments to remove seedlings or young plants from previously cleared areas, and this is likely to enhance the chances of permanently combating the weeds concerned (Moran *et al.*, 2004). A reduction in propagule pressure should also reduce the rates of spread of plants and reduce their ability to colonise new areas (Foster, 2001; Rouget and Richardson, 2003). Despite the fact that *M. servulus* populations take several years to build up to the point where seed-reduction becomes consistently high, and that dispersal rates are relatively slow, this study has shown that the weevils have the potential to make a positive contribution towards the biological control and management of *A. cyclops* seeds.

(*Footnote*: Research presented in Chapters 2 & 3 has been published as Impson *et al.*, 2003).

CHAPTER 4

IMPACTS OF FEEDING BY NATIVE ALYDID BUGS, *ZULUBIUS* SPECIES, AND BIRDS ON GERMINATION OF *ACACIA CYCLOPS* SEEDS

INTRODUCTION

A prolific production of long-lived seed characterizes many of the alien plant species that have become naturalized in South Africa. Many soil-stored seeds are capable of surviving in a dormant condition for several years, and an estimated 85% of the Fabaceae have water impermeable seed dormancy (Rolston, 1978). The mechanisms that control this dormancy within the acacias are varied and a number of studies have implicated seed characteristics, *i.e.*, size, shape, composition of the testa (Hanna, 1984; Morrison *et al.*, 1992), while others have focused on environmental and other factors such as scarification, various wet and dry heat treatments (including fire), use of acid and exposure to microwaves (Jones, 1963; Clemens *et al.*, 1977; Tran, 1979; Hendry and van Staden, 1982; Pieterse and Cairns, 1986a, b; Auld, 1986; Jeffery *et al.*, 1988). The effect of passage through the gut of various vertebrate species on germination of seeds is also well documented (Phillips, 1927; Janzen, 1971; Lamprey *et al.*, 1974; Noble, 1975; and Glyphis *et al.*, 1981).

The seeds of *A. cyclops* are borne in pods that develop in clusters. Once these pods split open during summer, the dark brown/black seeds are exposed and retained unlike many other *Acacia* species that shed their seeds soon after the pods dehisce. The seeds of *A. cyclops*, which can be held for up to six months on the plant, are surrounded by a bright red, fleshy arillate funicle (aril), a colour combination which has been demonstrated to be attractive to birds (Turcek, 1963). Glyphis *et al.*, (1981), recorded a number of potential avian dispersers of *A. cyclops* seed in South Africa and showed that passage of the seed through bird gut enhanced germination. This endozoochory, together with the high annual seed-production of the plant, has undoubtedly enhanced the successfulness of *A. cyclops* as an invasive species.

Several studies in South Africa, including aspects of this study, have shown that whilst exposed in the canopy-held pods, the ripe *A. cyclops* seeds are subject to attack by seed-sucking alydid bugs. Such feeding by hemipterans was not unexpected since

alydid adults and nymphs are known to be generalist feeders on seeds of leguminous plants (Holmes *et al.*, 1987a), and exploratory surveys in Australia had also recorded a number of species of hemipterans on *A. cyclops*. Of these, nine were either common or abundant, with one species, *Coleotichus costatus* (F.) (Scutellaridae), accounting for 25% seed damage (van den Berg, 1980c).

In the case of alydids, the feeding holes that pentrate the seed testa, enhance the seeds ability to imbibe water, and depending upon the severity of the feeding damage the swollen seeds will either germinate or rot (Neser, 1984). Holmes *et al.*, (1987a) and Holmes and Rebelo (1988), investigated the effect of alydid - (*Zulubius* species) feeding on the viability and germinability (*i.e.*, readiness to germinate in the presence of moisture, as opposed to dormancy) of *A. cyclops* seeds, and looked into alydid distribution across the range of *A. cyclops* in South Africa. The first of these studies showed that there is a corresponding decrease in seed viability with increasing alydid feeding-intensity, while the second study demonstrated that although germinability was not correlated with levels of alydid density and feeding damage, seed rotting during the germination trials was positively correlated with alydid damage. *Zulubius acaciaphagus* (Schaffner), which is the most abundant of the *Zulubius* species recorded from *A. cyclops* was also found to occur throughout the plants distributional range in South Africa, although it was most abundant in the hotter, drier areas, and in one such area was recorded as being capable of damaging up to 84% of the annual seed crop.

Although information was available regarding the impacts on germination of *A. cyclops* by these *Zulubius* species, in addition to the enhanced germination of seed having passed through bird gut, no studies had investigated the interaction between these two effects acting in combination. Feeding by alydid adults and nymphs on *A. cyclops* seed has no impact on whether the seed is shed from the plant, thus when both alydids and birds are present in an area, at least some of the seeds will be fed on by alydids prior to being removed from the pods by birds. The nature of the interaction between bird passage and alydid damage was considered to have potential implications for biological control of *A. cyclops* in South Africa. It was hypothesized that when birds feed on seeds that have been previously fed on by alydids, the net effect would be a higher reduction in dormancy (than would be seen with no previous

alydid feeding), and in turn an increase in the germinability of that seed. Thus, a study was undertaken to ascertain how combinations of these treatments might affect the viability and germination of *A. cyclops* seeds.

METHODS

Definition of terms

Viability is a measure of whether a seed is still alive (with no consideration of dormancy). Seed viability can be measured by clipping batches of seeds, (and thus breaking seed coat induced dormancy), and determining the number that germinate. Those that do not do so within six weeks are not viable and never grow.

Germinability is a measure of whether the seed is alive and not dormant (*i.e.*, it is ready and able to imbibe moisture and initiate growth). Germinability of seeds is measured by exposing entire seeds (*i.e.*, not clipped), to moisture and determining whether or not they imbibe and start to grow within eight weeks.

Dormant seeds refer to those seeds which may be either viable or inviable, but that do not germinate spontaneously despite conditions being favourable (*i.e.*, certain mechanisms are required to release such seeds from their dormant condition). In the case of *A. cyclops*, dormancy is imposed by the hard seed coat.

Viability & germination experiments

Several hundred seed-pods were collected from trees at Yzerfontein during March 2001 and transported back to the laboratory in bags with as little disturbance as possible. Seeds were carefully removed from the pods and inspected under a microscope for signs of alydid feeding turrets (*i.e.*, characteristic white extrusions of an unknown exudate, possibly liquefied, partially-digested cotyledon, that flows from and hardens in a turret shape around the penetration holes made by feeding alydids) (Plate 7). Feeding turrets were counted and seeds were then separated into categories according to the number of turrets they bore.

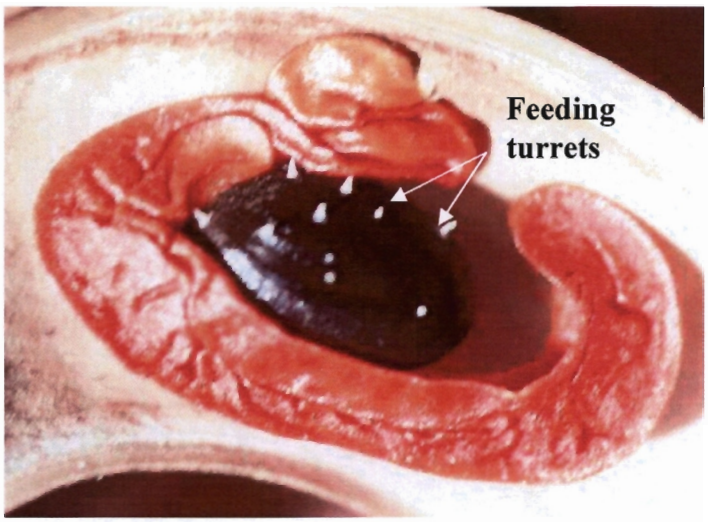


Plate 7. *Acacia cyclops* seed showing alydid feeding turrets

Prior to setting up the main germination experiment, two preliminary experiments were carried out to verify that the current procedures provided results that matched those from previous studies of Holmes *et al.* (1987a) and Holmes and Rebelo (1988).

a) *Breaking seed coat dormancy*

A series of 100 healthy seeds were used for trials to ascertain the best method for breaking dormancy. Of these seeds: 25 were clipped (*i.e.*, the testa was incised at the mycopylar end), and treated with a 6ml benomyl solution (a fungicide to protect against soil-borne pathogens); 25 were unclipped (*i.e.*, left complete and not cut at the mycopylar end), and not treated with an anti fungal agent (and hence were potentially exposed to soil borne pathogens); 25 were unclipped, and treated with a 6ml benomyl solution; and 25 were boiled and treated with a 6ml benomyl solution. The seeds were maintained in unsterilised moist sand in petri dishes in the laboratoryunder ambient conditions, and observed at least every third day over a six-month period for signs of germination. For all of the experiments carried out as part of this study, germination was considered to have taken place when the radical had developed to a length of 2mm or more (see also Pieterse and Cairns, 1986a).

b) *Effects of alydid feeding on the germination and viability of A. cyclops*

This experiment was set up to measure the effect of different densities of alydid feeding turrets on both the viability and germinability of *A. cyclops* seeds. Seeds

subject to these treatments fell into one of six categories; i) those with no turrets, ii) with one turret, iii) with two turrets, iv) with three turrets, v) with four turrets, and vi) with five or more turrets. Fifty seeds were used for each category. Half of the seeds from each category were clipped to measure viability, and the remaining seeds were left intact to measure germinability. All seeds were placed on moist sterilized sand for the duration of the trial. Seed trays were maintained at 25°C during the day and night with a 12h day:night photoperiod. The sand in the germination trays was sprayed with a fungicide and also kept moist with regular watering. Any of the unclipped seeds that had not germinated by the end of the trial were clipped and left for a further eight-week period in the germination trays, to determine whether they were viable or not. In some instances these unclipped seeds were imbibed and swollen, but no radicle had emerged and in most cases they were found to have rotted.

c) *The effects of alydid feeding and passage through bird gut on germination of A. cyclops seeds*

Once the above experiments had been completed, and a satisfactory method of initiating germination had been ascertained, a detailed laboratory study was set up in 2002. Seeds were placed into the following categories; a) seeds with no turrets, b) seeds with 1-2 turrets, c) seeds with 3-4 turrets, and d) seeds with ≥ 5 turrets. One hundred seeds were selected for each category. Eight red-winged starlings (*Onychognathus morio*) were then taken on loan, courtesy of the World of Birds (Hout Bay), and maintained in large walk in aviaries at the University of Cape Town. Half of the seeds (*i.e.*, 50) in each seed category were fed to pairs of starlings, in a mixed diet of fresh fruit and wheat cereal. The seeds were subsequently recovered from the droppings. Records were maintained of gut retention times. As soon as this part of the experiment was complete, the birds were returned to the World of Birds.

Of each of the groups of 50 seeds that had been digested by birds, half were clipped at the micropylar end to break the seed-coat induced dormancy, and hence test the viability, and the remainder were left un-clipped, to investigate the germinability. The remaining 50 seeds that had not been offered to birds were treated in the same way (Figure 4.1).

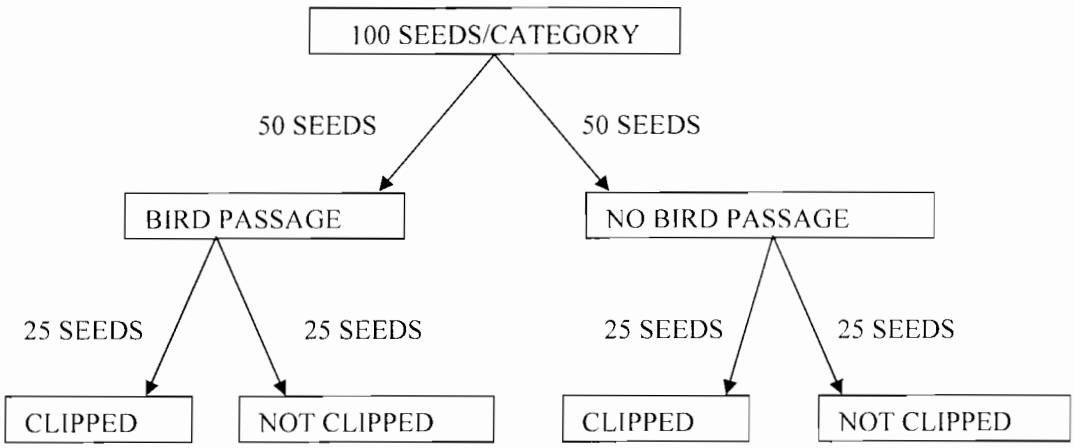


Figure 4.1. Diagrammatic representation of experimental design for each seed category used in the viability/germination experiment

For the laboratory trial, seeds from each category and treatment were placed singly in sieved, field-collected sand in polystyrene germination trays and placed in a phytotron unit. The temperature was maintained at a constant 25°C during the day and night with a 12h day:night photoperiod. The sand in the germination trays was sprayed with a fungicide and also kept moist with regular watering. The seedling trays were covered with a layer of clear plastic sheeting to keep the sand moist. Trays were monitored weekly for 77 weeks and details of seed germination recorded. Any germinated seeds were removed from the trays. The percentage of clipped seeds that germinated provided a measure of viability, while the percentage of unclipped seeds that germinated within eight weeks provided a measure of germinability. The proportion of seeds that were viable in the germinability experiment was calculated from the proportion (%) of seed that germinated in the viability trial.

Data analysis

Linear regression analyses were used to demonstrate (a) the correlation between alydid feeding intensity and numbers of seed germinated (and rotted), and (b) the additional impact of passage of the seed through bird gut on these factors. Analysis of Covariance (Statistica 7.0) on arcsin transformed percetage viability was used to compare the effects of bird and alydid feeding on the viability of *A. cyclops* seed (using number of feeding turrets as the co-variant). Yates corrected Chi squared tests

(Statistica 7.0) were used to determine the relationships between bird and alydid feeding on germinability of *A. cyclops* seeds.

RESULTS

a) Breaking seed coat dormancy

The preliminary viability and germination trials showed that the best method of breaking seed coat dormancy was to clip the micopylar end of the seed and 100% of the seeds germinated with this treatment. Healthy seeds, which were either unclipped or boiled, irrespective of treatment with anti-fungal agent, remained dormant.

b) Effects of alydid feeding on germination and viability of A. cyclops

There was a strong negative correlation (R^2 = 0.93) between alydid feeding intensity (number of turrets per seed) and seed viability (*i.e.*, germination of clipped seeds) (Figure 4.2a). When seeds were left unclipped, germination was low regardless of feeding intensity (Figure 4.2a), but there was a trend towards decreasing germinability in batches of seeds with either no damage or with high levels of damage. There was a positive correlation, (R^2 = 0.97), between alydid feeding intensity and seed mortality (Figure 4.2b).

Both of the preliminary germination experiments gave results that correspond with those of Holmes *et al.*, (1987a) and Holmes and Rebelo, (1988). The trials showed, that seed coat dormancy is easily broken by clipping the seeds, and that whilst alydid feeding may break seed dormancy allowing some seeds to germinate, a far higher portion of alydid-damaged seeds imbibe moisture, but fail to germinate and eventually rot. Hemipterans have been recorded as being vectors of fungi (Green and Palmbald, 1975), which might explain the high correlation between alydid feeding-intensity and seed rotting (Figure 4.2b). However, rather than being vectors of fungi, feeding by alydids may merely be allowing ingress of fungi into the punctured seeds.

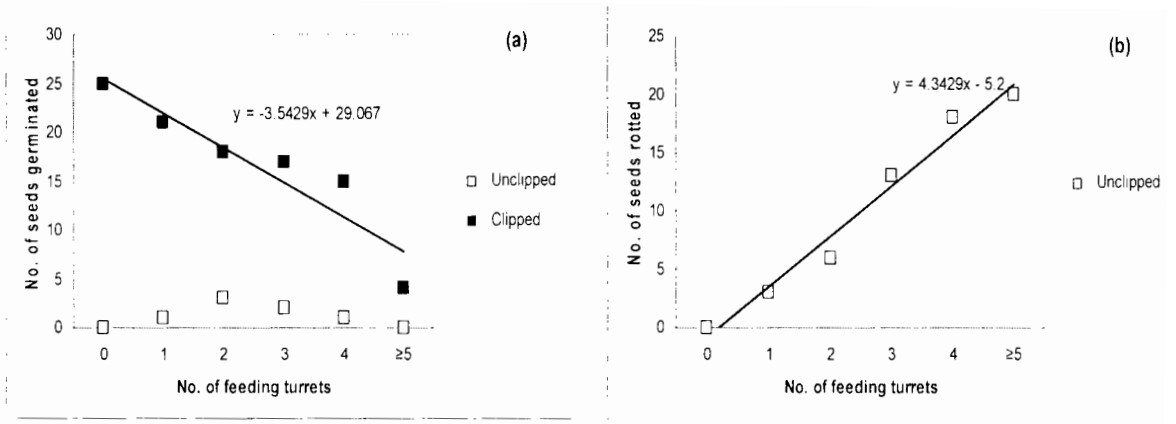


Figure 4.2. Germination (a) and rotting (*i.e.*, the non-dormant portion of seeds that imbibed but failed to germinate) (b) of unclipped *Acacia cyclops* seeds fed on at different alydid intensities.

c) The effects of alydid feeding and passage through bird gut on germination of A. cyclops seeds

Gut retention time of *A. cyclops* seeds by the captive red-winged starlings was mostly short and 84% of the seeds were passed within 24 hours of being eaten, while 98.6% were passed by day three, and the remainder by day six. Bird feeding had no impact on the viability of completely intact, healthy, seed. As was shown in the previous experiment (b), there was a strong negative correlation between feeding intensity and seed viability, however this was irrespective of whether seeds had passed through birds (R^2 = 0.8668), or not (R^2 = 0.7386) (Figure 4.3). An ANCOVA on arcsin transformed percentage viability showed that there were no significant differences between seeds that had not been eaten by birds compared to those that had been eaten ($F_{(1,5)} = 0.6948$, $P = 0.443$). However in both treatments, alydid feeding intensity had a significant impact on the viability of the seed ($F_{(1,5)} = 22.56$, $P = 0.005$).

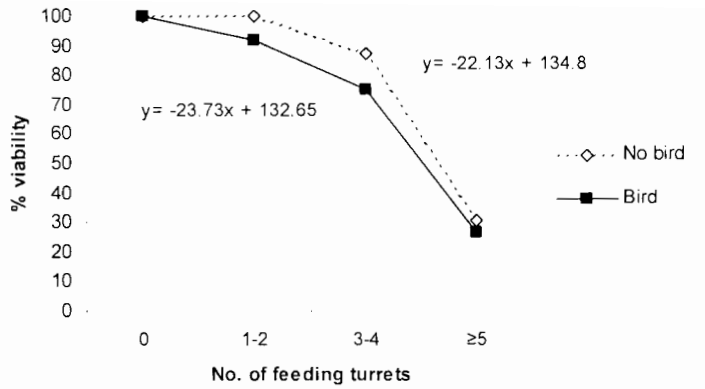


Figure 4.3. The effects of bird passage and alydid feeding-intensity on the viability of *Acacia cyclops* seed under controlled laboratory conditions.

The results of the germinability trial (Chi-squared analyses) showed that there were significant differences between the bird and no bird trials, with bird feeding significantly impacting on the germinability of the viable proportion of *A. cyclops* seeds (Figure 4.4). Alydid feeding intensity on the other hand, did not have any significant impact on germinability of seeds, supporting the data shown in figure 4.2a.

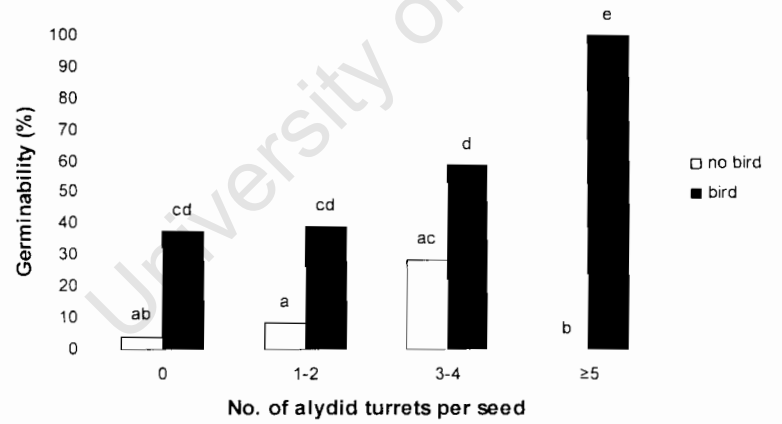


Figure 4.4. The influence of bird passage and alydid feeding-intensity on the germinability of *Acacia cyclops* seed. Letters above bars indicate similarities between data.

It is interesting to note however that unlike the situation in the no bird trial, bird feeding actually increased the germinability of seeds with five or more alydid feeding turrets. Despite an estimated 30% of the seeds (*i.e.*, seven of the 25 seeds) with more

than five alydid turrets being viable in the no-bird trial, no seeds germinated in the experiment.

From the trial, it was also possible to investigate the impact of bird feeding on seed germination over time. In order to simplify the presentation of the data, the analysis combined all the alydid feeding categories (from 0 to >5). Where seeds had been passed through bird gut, there was a clear increase in germination rates between week four and week six, supporting the hypothesis that bird passage reduces dormancy and thus enhances germinability causing a much larger proportion of the bird-digested seeds to germinate within 77 weeks (Figure 4.5). This is in contrast to the situation where no bird feeding has taken place, where there is a slow accumulation of germinated seed, and lower rates of germination.

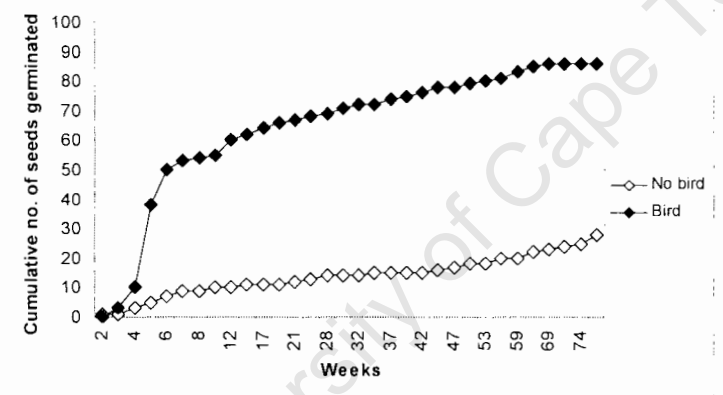


Figure 4.5. The effect of bird feeding and digestion on the germination of *Acacia cyclops* seed over time.

DISCUSSION

Under natural circumstances in South Africa, feeding on *A. cyclops* seed by birds or alydids or a combination of the two, is quite likely to occur. *Acacia cyclops* is well adapted for bird dispersal, and thus the seeds are able to cope with passage through bird gut. This study demonstrated that bird feeding enhanced germinability of *A. cyclops* seeds by breaking seed coat dormancy, and increased the likelihood of

early germination. Likewise, intensive feeding by alydids enhanced the germinability of the seeds but this was offset by a substantial reduction in the viability of alydid-damaged seed, and many of these seeds failed to germinate. Potentially, alydids could be having a detrimental effect on the health of seed banks of *A. cyclops*, particularly where alydid feeding-intensity is high (five or more turrets per seed). However where alydid feeding-intensity was relatively low (one to four feeding turrets per seed), germinability increased relative to undamaged and heavily damaged seeds.

Although the potential effects of alydid feeding and bird passage on *A. cyclops* seed have been demonstrated, alydid abundance and feeding intensity may vary both spatially and temporally in *A. cyclops* stands. The frequency of removal of ripe seed by birds may also vary, and has not been quantified. Holmes and Rebelo (1988) showed that there was considerable variation in alydid feeding intensity both within, and between sites. It is also believed that alydid populations build up from late spring into the summer, so those seeds which mature slightly later could be prone to higher levels of feeding by these insects (Gill, 1985). Seeds that mature early and remain hanging in the pods on the plant are also subject to increased alydid damage over time. There is little known about the impacts that alydids and/or birds are having on seeds produced by isolated *A. cyclops* plants, or in young stands, where seed production is likely to be lower per square metre than in the mature stands. Studies thus far have focussed on dense mature stands and have been carried out over a short term.

The real impacts that birds and alydids are having in the natural situation can only be realised through a better understanding of the distribution and population dynamics of the *Zulubius* species, and the various avian seed dispersers. Whatever these impacts may be, *A. cyclops* populations are unlikely to be measurably affected by alydid feeding alone. However the effect of birds (either in combination with alydids or alone) on the dynamics of *A. cyclops* is potentially of greater concern. Not only do birds enhance the germinability of *A. cyclops* seed, but they also disperse the seed allowing colonisation into areas where there are "safe sites" (c.f. Harper, 1977), and the seeds can then be recruited into existing or new populations of the weed.

CHAPTER 5

INTERACTIONS BETWEEN NATIVE, SEED-FEEDING ALYDID BUGS, *ZULUBIUS* SPP., AND THE INTRODUCED BIOCONTROL AGENT, *MELANTERIUS SERVULUS*, ON *ACACIA CYCLOPS*

INTRODUCTION

In addition to overseas surveys to identify potential suitable agents for biological weed control, observations of the target weed within the country of invasion should ideally be undertaken, to determine whether native insects and pathogens might be exerting some form of pressure on the weed. Surveys of *A. cyclops* in South Africa have shown that there are several vertebrate and invertebrate seed-feeders on the plants. Rodents probably play the most important role in seed removal and destruction, in particular the striped fieldmouse, *Rhabdomys pumilio* (Sparrman), and the gerbil, *Tatera afra* (Gray) (David, 1980), which feed on ripening and mature *A. cyclops* seed. Two species of doves, *Streptopelia senegalensis* (L.) and *S. capicola* (Sundevall), the Cape weaver, *Xanthophilus capensis* and the Bully seedeater *Serinus sulphuratus*, have been recorded feeding on mature *A. cyclops* seed (Winterbottom, 1970; Glyphis *et al.*, 1981). Several other bird species, in particular the red-winged starling, *Onychognathus morio*, are attracted to the nutritious bright red aril surrounding the seed, and although they feed on the aril and seed, the seed passes through the gut unharmed, and enhances subsequent germination (see Chapter 4).

As far as invertebrate seed-feeders are concerned, an unidentified species of tortricid moth (Tortricidae: Olethreutinae), has been recorded from *A. cyclops* seeds (Donnelly and Stewart, 1990) and both adults and nymphs of an unidentified pentatomid (possibly *Antestiopsis orbitalis*), have been observed feeding on the green ripening seed pods of the plant (personal observations, see Chapter 3). There are also a number of species from at least four genera within the Alydinae (Hemiptera: Alydidae) that occur on both exotic and indigenous acacias (Schaefer, 1980). At least one genus, *Zulubius*, is present on *A. cyclops*, with the species *Zulubius acaciaphagus* (Schaffner, 1987), being the most abundant. Since alydids commonly occur on the

seeds of native leguminous plants in South Africa, those that now occur on *A. cyclops* are believed to have moved onto this food source from one or more indigenous plant species occurring nearby (Holmes *et al.*, 1987a).

The locally-occurring *Zulubius* species are temporally and spatially too sporadic to be able to provide alone a reliable supplementary role in reducing the invasiveness of *A. cyclops*. In fact substantial control of weed species through the action of indigenous insects or pathogens, although not impossible, (Cruttwell McFadyen, 2000; Edwards *et al.* 2000; De Clerck-Floate *et al.*, 2000), is certainly not common (Julien *et al.*, 1984).

Alydids are present on *A. cyclops* trees from October through to June, during the period when seeds are available to them to feed on, and are most abundant from January to March (see Chapter 3). Adult alydids enter the pods through splits that develop as the pods begin to ripen and dehisce (in October), and the females lay their eggs in clusters on the internal walls of the pods (personal observations). The alydid nymphs that emerge soon afterwards, feed on the hardened, brown seeds, leaving the characteristic small white turrets that mark the feeding sites of both adult and nymphal stages.

Although a certain amount of information was already available regarding the type and extent of damage that *Zulubius* species cause to *A. cyclops* seeds (Chapter 4), and when and where the alydids occur on the plants, nothing was known about how the indigenous alydid insects might impact or interact with *M. servulus*.

Zulubius species utilise *A. cyclops* seeds that have ripened and turned hard. *Melanterius servulus*, on the other hand exploits the resource at an earlier stage and only utilises the green ripening seeds before the testa turns hard. Thus although there is no direct competition for seeds between the two groups of insects, the feeding activity of *M. servulus* determines the abundance of seeds that eventually become available to the alydid bugs. Besides the direct damage caused by the weevils, *M. servulus* may have an indirect effect on the availability of seeds for the alydids. Adult and larval feeding by the weevils causes general pod degradation, which prevents the seed pods from opening properly at dehiscence, so that even healthy

seeds remain confined and inaccessible within the pods (personal observations). The consequent decline in absolute numbers of available seeds might affect the alydids in a number of ways. Of special interest was the possibility that levels of damage caused by alydids might increase after the release of *M. servulus*. It was hypothesized that if population levels of alydids remain stable in areas where *M. servulus* is active, the number of seeds available per alydid will decline, resulting in greater levels of damage per seed. A series of surveys were carried out to determine how the introduction of *M. servulus* has affected the alydids and the levels of damage they cause on *A. cyclops*.

METHODS

Three *A. cyclops* sites were selected to monitor damage by *Zulubius* spp. Samples were taken from the center of the sites, where seed-damage levels by *M. servulus* were known to be high, and at an equivalent point where *M. servulus* was scarce or non-existent. On the western coastal plain, high and low damage sites were selected at both Langebaanweg and Yzerfontein. On the eastern coastal plain, the Potberg and Breede River sites were paired as the high *M. servulus* damage and *M. servulus*-free sites respectively even though the two sites were much further apart (approximately 15km) than the paired sites on the western coastal plain. The sites were used for other aspects of this study (see Chapters 2 and 3) and are illustrated in Chapter 1, Figure 1.3.

Each month from November 2000 to June 2001, branches bearing mature seed-pods were collected indiscriminately from ten different trees at each of the representative sites. (due to an oversight, no sample was taken from the 'low' *M. servulus* site at Yzerfontein during January 2001). The quantity of plant material collected from each tree was standardized, by using large plastic bags (520 x 470 cm) that were opened and, as quickly as possible, placed around a cluster of branches. The open end of the bag was gathered around the stems of the branches, which were then cut. The cut stem ends were pushed down into the bags, which were sealed and transported to the laboratory where they were kept in a freezer (at -18°C) until being scrutinized.

Each sample bag was opened separately and a count was made of all the alydid adults and nymphs, as well as *M. servulus* adults trapped within. The pods were removed from the branches, counted and replaced in the bags before a sub-sample of ten pods was removed, unsighted, from each bag. Seeds within each of the selected pods were then assessed for *M. servulus* damage (see Chapter 3), and remaining, 'healthy' seeds were examined under a microscope in order to make counts of alydid feeding turrets (Plate 7, Chapter 4), on each of the seeds.

An attempt was made to quantify alydid feeding on seeds that had been shed from the canopy at each of the sampling periods. This was done by placing a circular wire measure, (30cm in diameter), on the ground under trees that were bearing seed pods. The leaf litter and seeds from the surface layer within the circular measure were collected, and any alydids and seeds were removed and counted, and seeds were inspected for signs of feeding turrets.

Data analysis

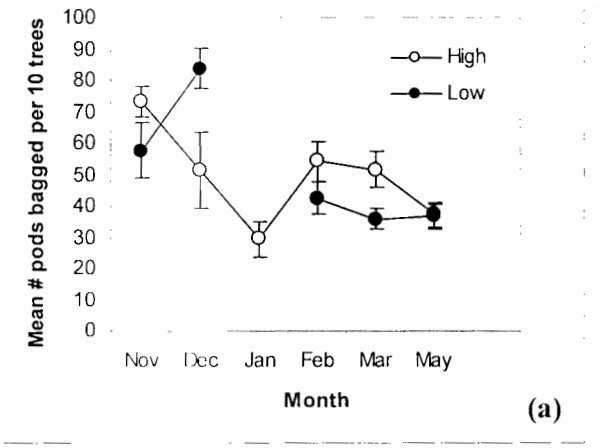
To clarify that pod abundance did not vary significantly at sites comparing low and high *M. servulus* damage, factorial analysis of variance was used (using site and density as the variables), (Statistica 7.0). Factorial ANOVA (using site and density as the categorical factors and available seed as the dependent variable) was also used to compare the amount of healthy seed available to alydids at each of the sites for all of the months sampled. Main effects ANOVA on arcsine transformed data of percentage seed damage was used to compare the amount of seed utilized by alydids at the low and high *M. servulus* sites.

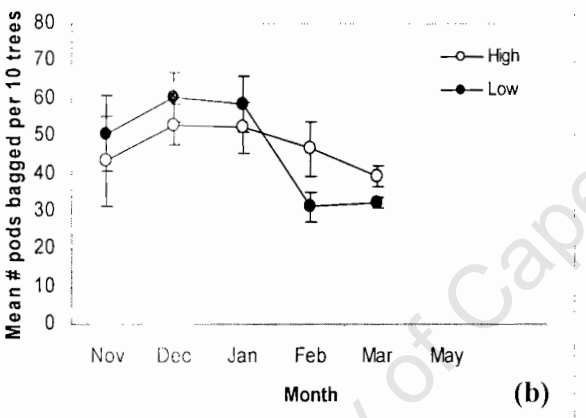
RESULTS

The experiment to investigate alydid numbers and feeding on the *A. cyclops* seed on the ground did not achieve its anticipated goal. Firstly, the feeding turrets are fragile, and easily broken off the seeds, and consequently there were no turrets on the approximately 80,000 ground-collected seeds. Secondly, although alydids are occasionally observed on the ground, only one alydid was ever collected in a sample, indicating the insects do not frequent the leaf litter.

Despite the fact that there was some variation in pod abundance (and hence numbers of seeds available) between trees at each of the sites, a factorial analysis of variance showed that the pod abundance per site (*i.e.*, from bagging ten trees) was not significantly different between the selected 'high' and 'low' *M. servulus* areas at Yzerfontein ($F_{(1,98)}$ = 0.725, $P = 0.396$) and Langebaanweg ($F_{(1,90)} = 0.005$, $P = 0.94$), (Figures 5.1 a and b). This was not the case for the two sites on the east coast where there were significantly more pods in the 'low' weevil-damage area than in the 'high' area, ($F_{(1,208)} = 9.46$, P = 0.002), (Figure 5.1c).

There were significant differences in numbers of pods collected on different dates at Yzerfontein, (ANOVA, $F_{(5,98)} = 9.92$, $P < 0.001$) and Langebaanweg, (ANOVA, $F_{(4,90)} = 3.53$, P = 0.01), however this was not the case for the east coast sites, (ANOVA, $F_{(5,208)}$ = 0.395, $P = 0.85$). Despite the differences in pod numbers at the east coast 'low' and 'high' areas, the greater number of pods at the 'low' area, allowed for the original hypothesis (*i.e.*, more pods and hence seeds being available to alydids) to be tested. Disparities in patterns of pod abundance between the three main sites necessitated that each site was analysed separately.





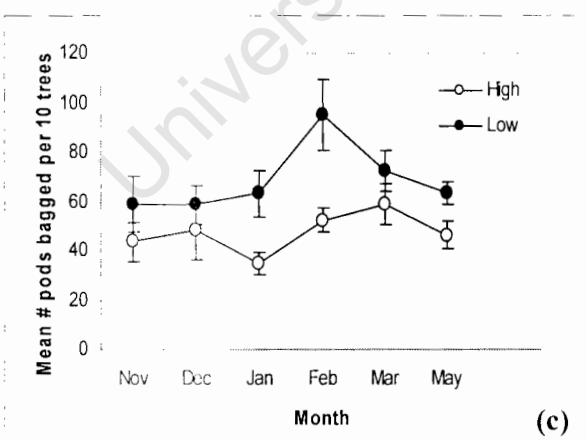


Figure 5.1. Mean (± standard error) number of *Acacia cyclops* pods collected from ten trees through the year at 'low' and 'high' *Melanterius servulus* sites at (a) Yzerfontein, (b) Langebaanweg and, (c) east coast.

The total counts of alydid adults and nymphs from the bagged samples showed considerable variability indicating that alydids were patchy in their distribution and generally low in abundance (Table 5.1). Although the method gave an indication of presence or absence of alydids within the area at a particular time, it was considered to be too unreliable for assessing alydid density and to use as a comparative tool with the seed damage analysis.

Table 5.1. Numbers of alydid adults and nymphs collected monthly from the ten *Acacia cyclops* trees at each of the study sites (H – 'high' *Melanterius servulus*, L – 'low' *M. servulus*).

SITE	Alydids	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Jun
E. coast (H)	Nymphs	0	0	11	2	11	13	0	
E. coast (H)	Adults	1	0	0	1	2	1	1	
E. coast (L)	Nymphs	0	0	2	4	4	32	1	
E. coast (L)	Adults	0	0	0	0	4	8	0	
Yzerfontein (H)	Nymphs	0	0	1	5	16	10	0	0
Yzerfontein (H)	Adults	0	0	0	1	5	4	0	0
Yzerfontein (L)	Nymphs		0	9		19	5	11	2
Yzerfontein (L)	Adults		0	4		6	1	2	0
Langebaanweg (H)	Nymphs	0	0	0	0	1	1		
Langebaanweg (H)	Adults	0	0	0	0	0	0		
Langebaanweg (L)	Nymphs		0	8	4	2	0		
Langebaanweg (L)	Adults		0	0	1	0	0		

Seed damage levels attributable to *M. servulus* (Figures 5.2 a-c) were significantly higher in the selected 'high' weevil areas compared to the 'low' weevil areas at all three of the selected sites.

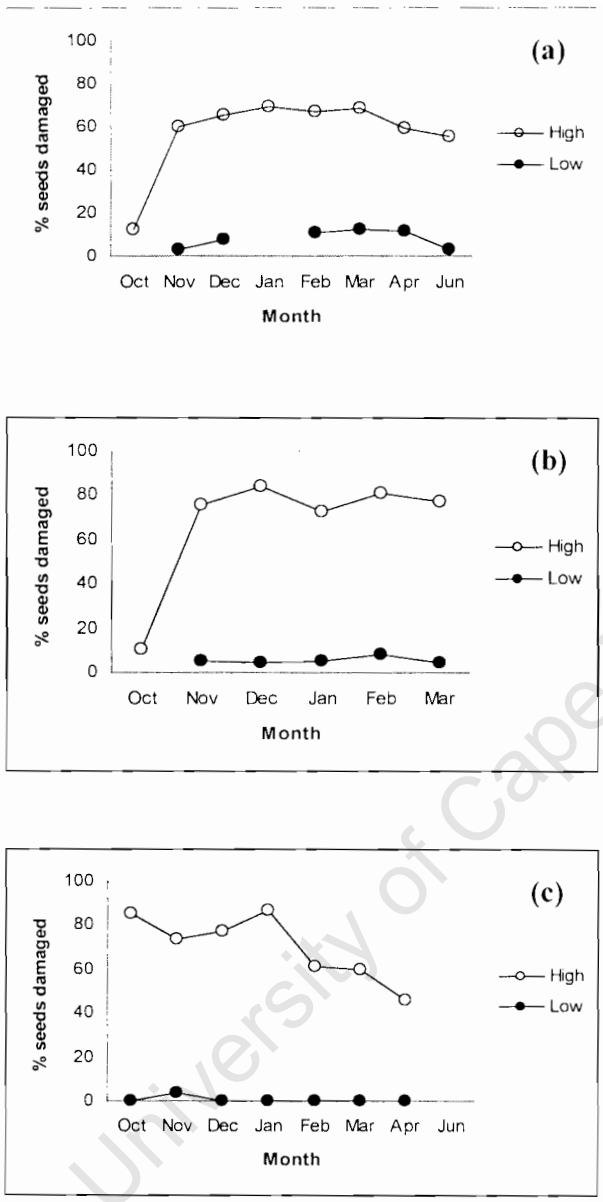


Figure 5.2. Seed damage levels attributable to *Melanterius servulus* at 'high' and 'low' areas at (a) Yzerfontein, (b) Langebaanweg, and (c) east coast.

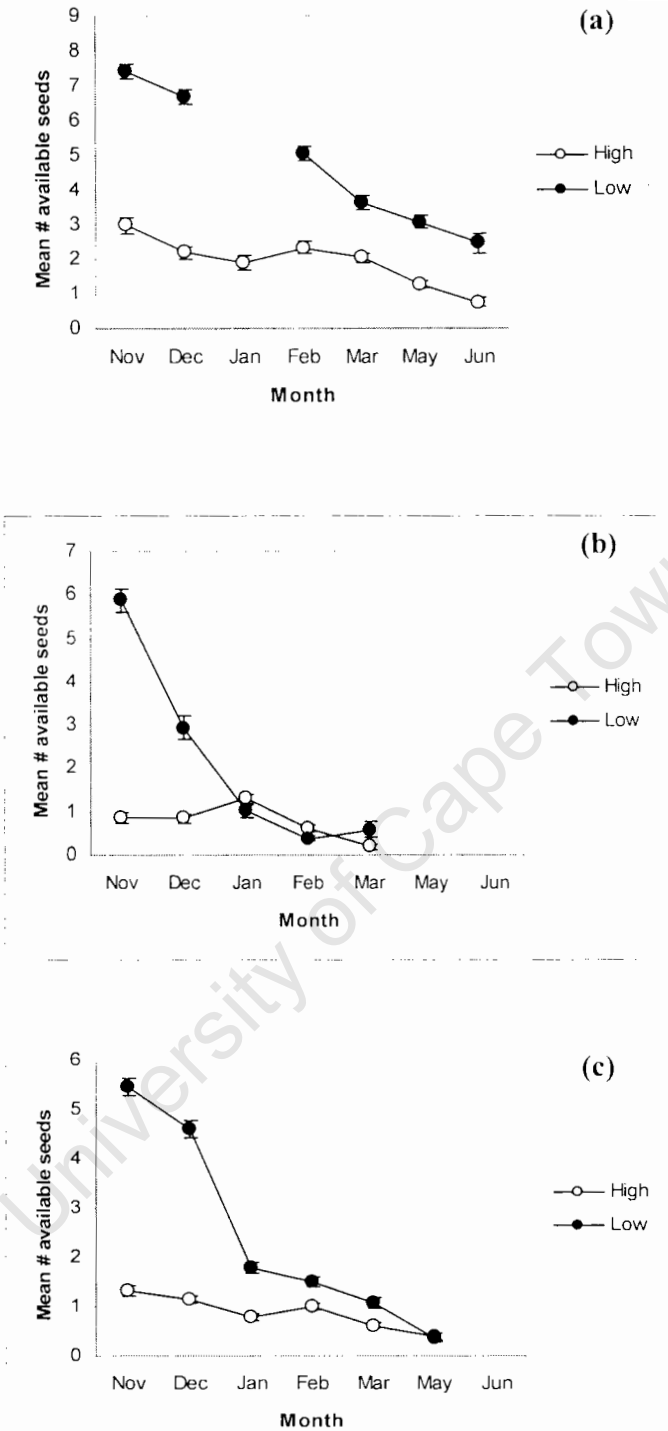


Figure 5.3. Mean (± standard error) number of healthy seeds per pod available for use by alydids at 'high' and 'low' *Melanterius servulus* sites at (a) Yzerfontein, (b) Langebaanweg, and (c) east coast.

Comparisons of the numbers of healthy seeds available for use by alydids at each of the high and low *M. servulus* sites are shown in Figures 5.3 a-c. A factorial analysis of variance confirmed that there were highly significant differences between numbers of seed available at Yzerfontein ($F_{(1,1085)}$ = 545.4, P <<0.0001), Langebaanweg ($F_{(1,987)}$ = 193.37, P << 0.0001), and the east coast sites ($F_{(1,2178)}$ = 396.97, P << 0.0001). In all cases more seeds were available for use by alydids at the 'low' *M. servulus* sites, particularly at the critical time when the alydid adults would have been laying their eggs towards mid-summer (November and December).

Melanterius servulus adults utilise the fully developed, green, *A. cyclops* seed to oviposit on during September/October, which explains the reduced number of available seeds after this time at all the 'high' sites. Towards the end of the study period, both 'high' and 'low' sites reflected similar numbers of available seed, due to the fact that by this time healthy seed had started dropping or had been removed from the canopy held pods at all sites. This pattern was enhanced by the fact that pods damaged by *M. servulus* often remain closed and healthy seeds are trapped, hence the numbers of healthy seeds in samples from the 'low' and 'high' weevil areas begins to equalize.

At Yzerfontein (Figure 5.4), at the start of the summer season (November), none of the available seeds were fed on by alydids. However as summer progressed seeds were utilised in both 'high' and 'low' areas. Using main effects ANOVA on arcsine transformed data, significantly more seeds were utilised by alydids at the 'low' *M. servulus* site ($F_{(1,5)}$ = 7.69, P = 0.03), than at the 'high' site.

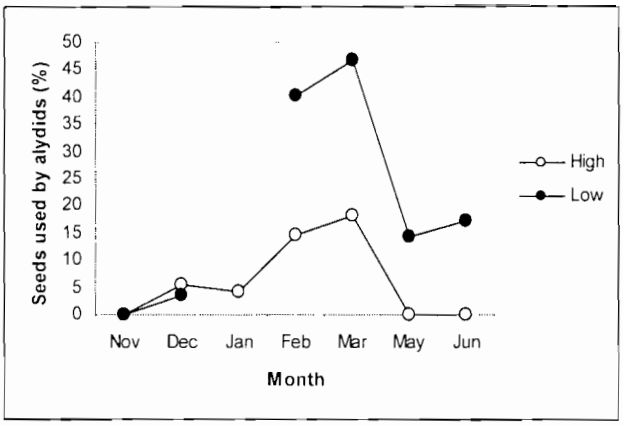


Figure 5.4. Percentage of available seeds used by alydids in 'high' and 'low' *Melanterius servulus* areas at Yzerfontein.

Similar analyses for Langebaanweg (Figure 5.5) also showed significantly more seeds being used by alydids at the 'low' *M. servulus* site ($F_{(1,5)} = 8.3$, $P = 0.03$). Overall, very low numbers of alydids (and hence corresponding damage levels) were observed and collected from Langebaanweg, particularly at the 'high' *M. servulus* site.

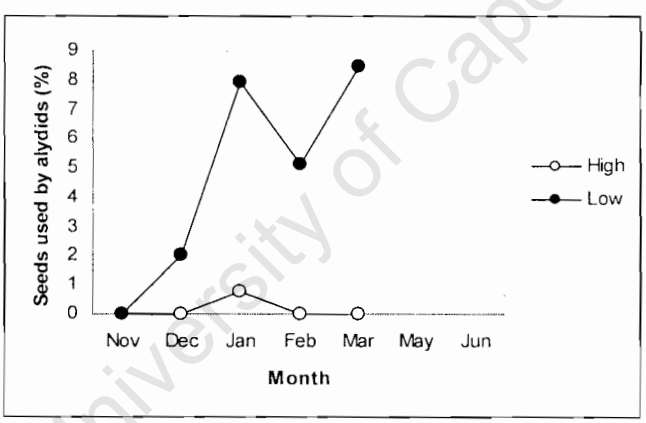


Figure 5.5. Percentage of available seeds used by alydids in 'high' and 'low' *Melanterius servulus* areas at Langebaanweg.

A comparison of the numbers of seeds utilised by alydids at the 'high' and 'low' east coast sites showed no significant differences between the two areas (Figure 5.6), ($F_{(1,5)} = 0.017$, $P = 0.9$).

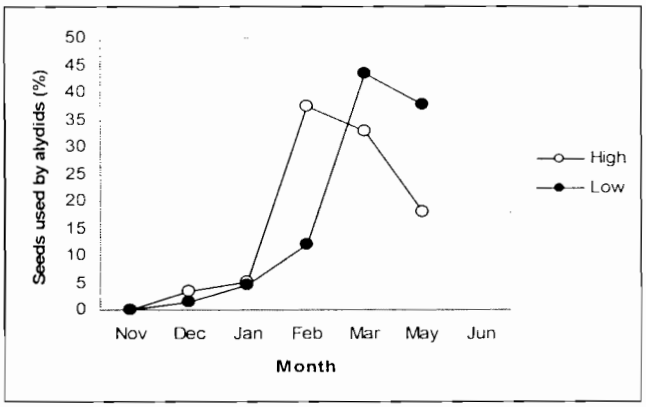


Figure 5.6. Percentage of available seeds used by alydids in 'high' and 'low' *Melanterius servulus* areas at the east coast.

The highest usage of seeds was recorded in March at Yzerfontein where *M. servulus* levels were low (Figure 5.4), and then at the east coast (Figure 5.6), where respectively, 46% and 44% of the available seeds had feeding turrets. However the average number of seeds that were used by alydids during the season was less, with 17% and 6.9% respectively at the low and high *M. servulus* sites at Yzerfontein, 1.9% and 0.3% respectively at the low and high sites at Langebaanweg, and 6.4% and 13.7% at the low and high east coast sites. At all three of the sites the percentage of available seeds that had turrets increased between December and March (Figures 5.4, 5.5, 5.6). However, subsequent to this, the level of usage of available seed dropped at both Yzerfontein (Figure 5.4), and the east coast (Figure 5.6) rather than increasing as might have been expected. Once again this was probably due to the fact that available seeds were not limiting and also that alydid populations had declined before the late seed ripened. At Langebaanweg, no seeds were left on the trees in May, and thus there is no record of the numbers of seeds used by alydids.

Although the results showed a trend towards higher levels of alydid feeding damage where *M. servulus* levels were low, (and thus increased numbers of seed were available), it was necessary to ascertain whether this translated into higher feeding pressure (*i.e.*, number of turrets) per seed, and whether there was any variation from the beginning to the end of the season. This was considered to be important because

levels of five or more turrets per seed prevented germination of much of the seed in this category (see Chapter 4).

Counts from all three of the sites showed no apparent changes in the number of alydid turrets per seed as the season progressed (Figures 5.7 a-c), despite a decline in the numbers of 'available' seeds on the *A. cyclops* trees. Even with this decline there were still many un-utilised seeds that were available to the alydids on the trees. Thus, availability of seeds is unlikely to be a limiting factor for alydids.

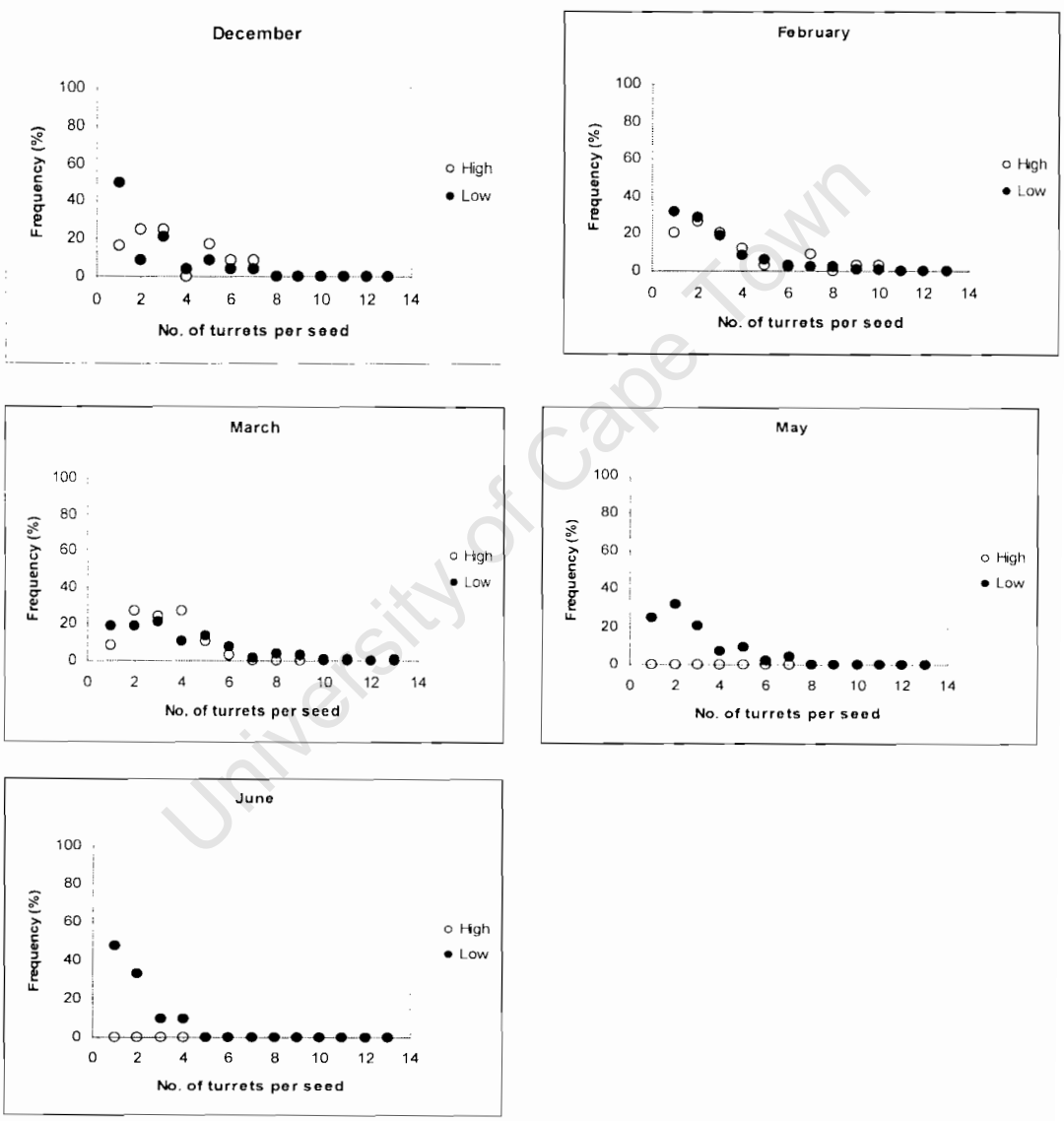


Figure 5.7.a The frequency of *Acacia cyclops* seeds with turrets (%), and numbers of turrets per seed as measured from December to June at the 'high' and 'low' *Melanterius servulus* areas at Yzerfontein.

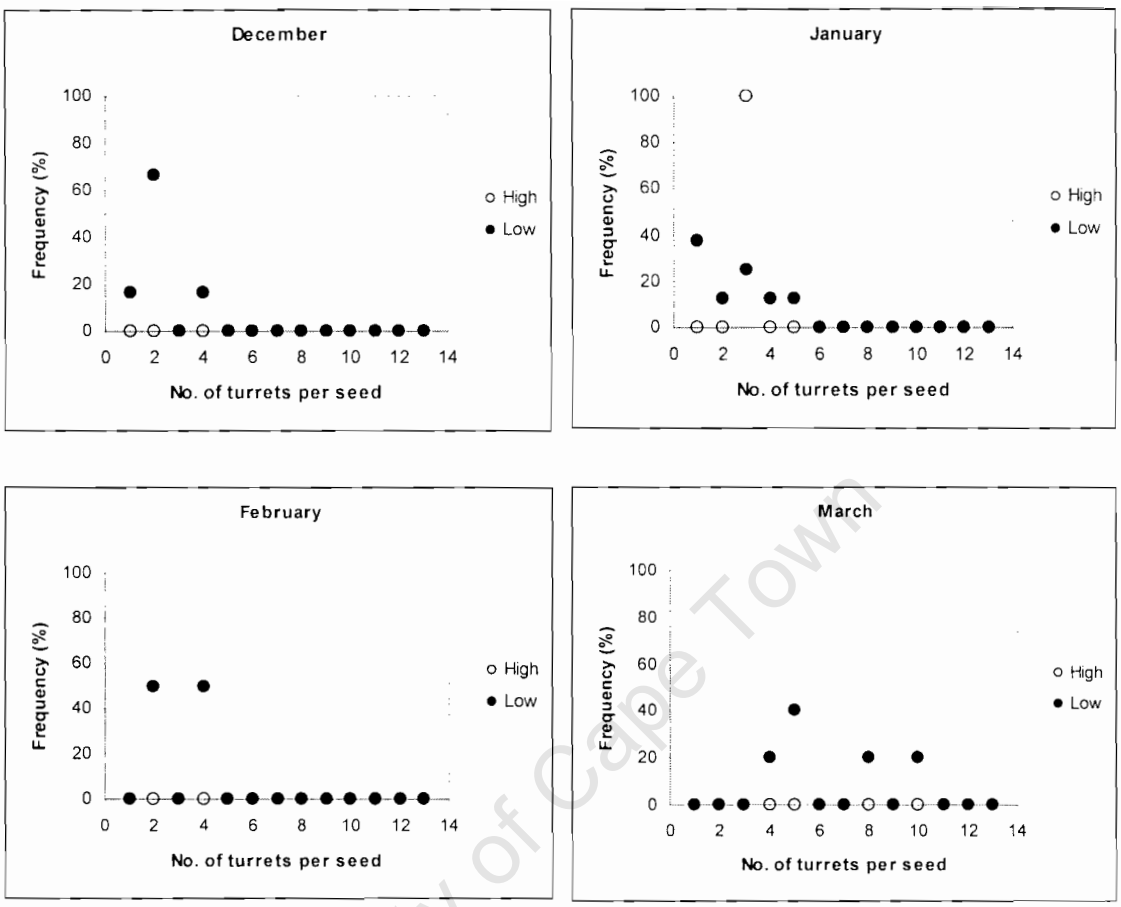


Figure 5.7.b The frequency of *Acacia cyclops* seeds with turrets (%), and numbers of turrets per seed as measured from December to March at the 'high' and 'low' *Melanterius servulus* areas at Langebaanweg.

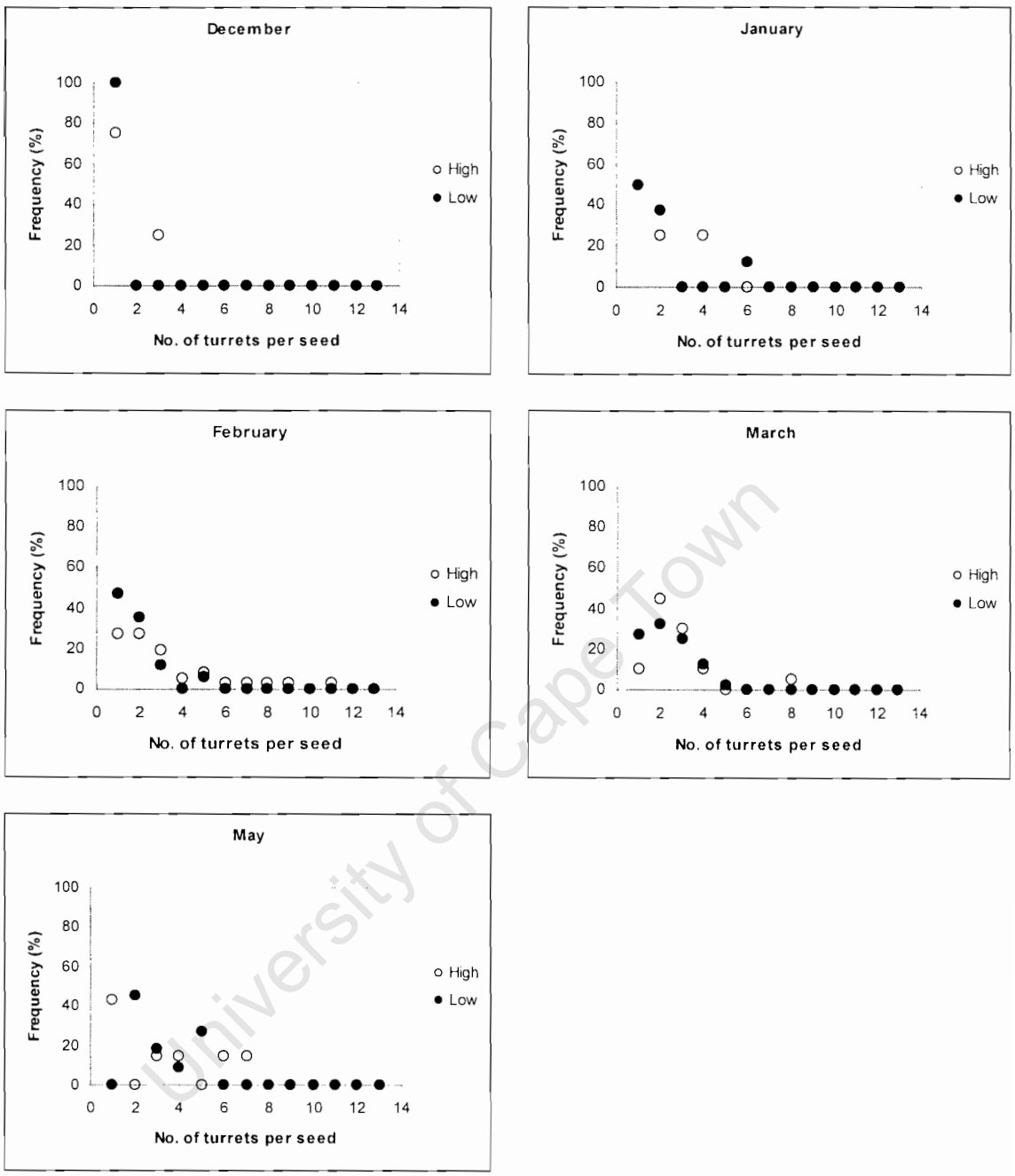


Figure 5.7.c The frequency of *Acacia cyclops* seeds with turrets (%), and numbers of turrets per seed as measured from December to May at the 'high' and 'low' *Melanterius servulus* areas on the east coast.

At Langebaanweg, there was only one record of alydid feeding in the 'high' *M. servulus* area during January (Figure 5.7b), and at no other time was alydid feeding recorded from this area. At all of the sites, the majority of the seeds utilised by alydids had between one and three feeding turrets per seed. Although these low levels of alydid feeding can reduce the viability of the seed, and increase the chances of failed germination, it has also been shown that a percentage of these seeds germinate, particularly with the added impact of bird feeding, and passage of the seed through bird gut (see Chapter 4). The germination experiment demonstrated that only much higher levels of alydid feeding (*i.e.*, more than five turrets per seed) had any real impact on the reduction of viability and germinability of the seeds. The proportion of seed with five or more turrets per seed was relatively low at all of the sites (Figures 5.8 a-c).

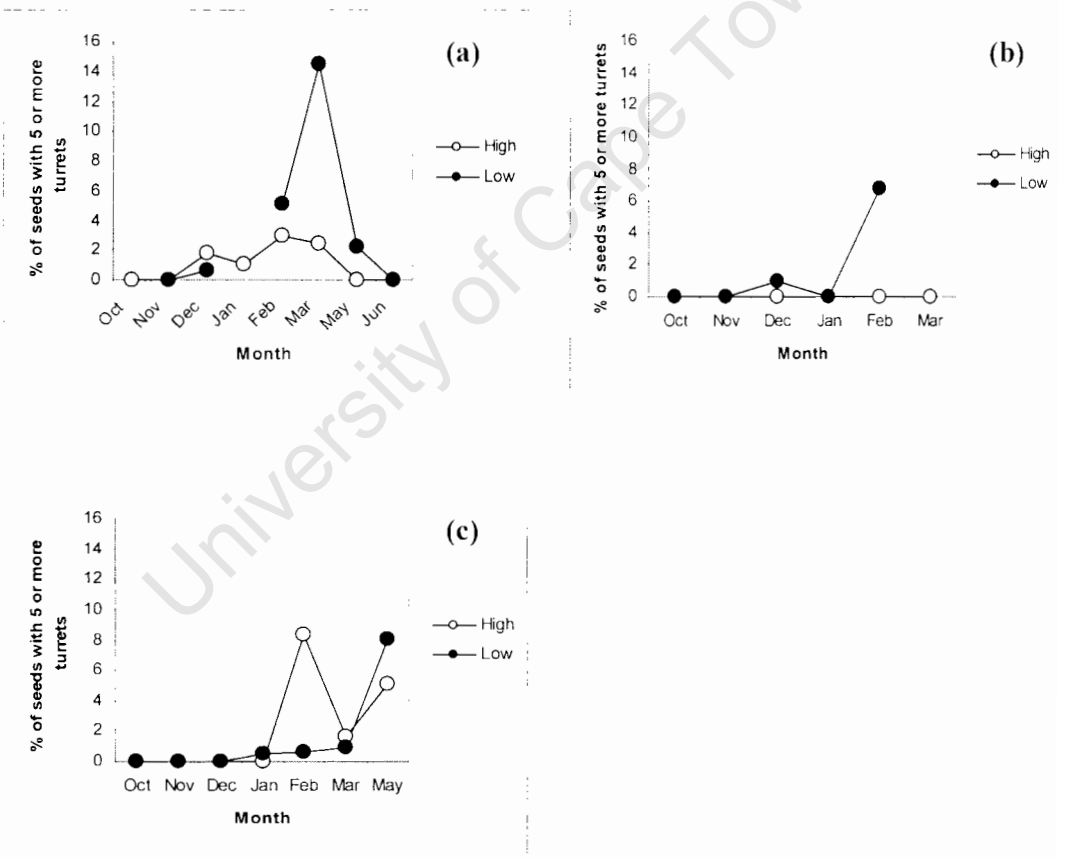


Figure 5.8. Percentage of seeds with five or more turrets per seed at (a) Yzerfontein, (b) Langebaanweg, and (c) east coast.

For all three of the sites, February and March were the two months during which the highest levels of alydid feeding per seed were recorded, however, overall throughout the season a relatively low percentage of seeds had five or more turrets at any of the sites

DISCUSSION

Although the evidence presented here shows that *M. servulus* could be impacting on alydid abundance in areas where the introduced biocontrol agent is abundant and causing high levels of damage, the overall impacts may only be realized once the beetles become established across a wider range and over a longer time period. Apparently the interactions between the two insect taxa do not have any noticeable negative consequences for the biological control of *A. cyclops*. Several studies have demonstrated wide variation, both at the site level and between sites, in pre-dispersal seed attack by alydids (Auld, 1983; New, 1983; Holmes and Rebelo, 1988). Owing to the inconsistent and sporadic nature of alydid presence and attack, the interactions between the introduced and indigenous insects are quite likely to vary from one year to the next.

The possibility of alydid-feeding supplementing biological control of *A. cyclops* seems to be limited due to the low levels of utilization of available seed by this group of insects. Although alydid damage reduces the viability of *A. cyclops* seed and increases seed rotting, particularly when feeding levels are high, it is unlikely that the alydids are having any measurable effect on the population dynamics of the plant. Annual seed-production by the plant is very high, and consequently large amounts of seed are not damaged and accumulate in a seed bank (Cloutier and Watson, 1990; Myers *et al.*, 1990). Under the circumstances, the necessity for a dependable, and more destructive, biological control agent was unquestionable.

Despite the fact that alydids can, in some circumstances (*i.e.*, high densities and intensive feeding), play a small supplementary role to *M. servulus* by reducing the viable seed-crop of *A. cyclops*, they are not consistent and thus the introduction of the biological control agent, *M. servulus* was well founded. The nature and timing of the

utilization of *A. cyclops* seed by this insect and indigenous alydids have meant that there are no direct, or potentially competitive, interactions between the two taxa. Furthermore, unlike the alydids, *M. servulus* populations remain consistently present at (and spread from), release sites from one year to the next. A pattern has also emerged showing that seed-damage levels at these sites increase over time, and will probably continue to do so until they reach a point where the populations of weevils stabilize.

SYNOPSIS

The invasion of the fynbos biome by alien vegetation has been of concern for almost a century (Woods, 1957; Taylor, 1962; Hall and Boucher, 1977; Macdonald *et al.*, 1986). *Acacia cyclops*, which is considered to be one of the main environmental weeds within this sensitive biome (Macdonald and Jarman, 1984), was targeted for biological control some 30 years ago (Neser and Annecke, 1973). Despite the fact that the presence of dense infestations of *A. cyclops* threaten biodiversity in the fynbos (Holmes, 1989; Esler and Boucher, 2004) and cause an increased fire risk (Stock and Allsopp, 1992), the economic importance and widespread use of this plant as a source of firewood, was of crucial consideration in the selection of potential biocontrol agents (Impson and Moran, 2004). The selection of potential agents thus focused primarily on phytophages that reduce the reproductive capacity of the plant, whilst not damaging the vegetative parts of the plant and compromising its economic use.

Seed-reducing biological control agents have for many years been the subject of discussion and debate, and from the early days of biocontrol programmes many practitioners have not been proponents for their use (Huffaker, 1964; Harris, 1973; Goeden, 1983). At least theoretically, such agents need to consistently destroy a high proportion of the seasonal seed crop in order to reduce the density and rate of spread of woody invasive weeds, particularly in situations where large numbers of long-lived seed are produced (Noble and Weiss, 1989; Hoffmann and Moran, 1991; Sheppard *et al.*, 1994; Kriticos *et al.*, 1999). Models based on studies of scotch broom (*Cytisus scoparius*) showed that a reduction in the reproductive capacity of the plant could reduce its density if the disturbance rate was high, and plant fecundity and seedling survival low (Paynter *et al.*, 1996; Rees and Paynter, 1997). These latter characteristics are not attributes of broom, and it was concluded that seed-feeders would be unlikely to reduce populations of the plant (Paynter *et al.*, 1996). Other studies have indicated that even with moderate to high levels of seed reduction, population densities of the weed are not necessarily reduced, and may, in fact, even increase (Powell, 1988; Myers *et al.* 1990; Kelly and McCallum, 1995; Hoffmann and Moran, 1998; Myers and Risley, 2000). Thus, the debate over the effectiveness of seed-reducing agents continues, and perhaps each programme needs to be considered

and evaluated in isolation, rather than making generalizations about seed-reducing agents as a whole.

The economic implications (*i.e.*, the costs of clearing and reducing spread and also costs to the environment in terms of water loss and biodiversity) of invasive plants are increasingly being recognized, and evaluation studies, which are a critical aspect of such cost-benefit studies, emphasize whether particular control methods and agents are adequate, or whether more are needed. Cost-benefit ratios are increasingly being drawn on when assessing whether particular weed control measures are justified, and in all cases the benefits of biological control greatly outweigh the costs of the weed (Coombs *et al.*, 1996; van Wilgen *et al.*, 1996; de Wit *et al.*, 2000; McNeely, 2000; McConnachie *et al.*, 2004).

Although in many situations, and particularly where seed-feeders are concerned, it may be difficult to evaluate the benefits of a biological control agent, simulation models may be useful in predicting the potential of an agent (Withers *et al.*, 2004) and also the rates of spread of invasions (Higgins *et al.*, 1996; Clark *et al.*, 2003) as well as the impacts of invasions on biodiversity (Higgins *et al.*, 1997). By using such tools estimations regarding the economic consequences of alien trees can be made.

In addition to modelling and evaluation studies in biological control programmes using seed-feeders, the question of what is meant by "effective" control should be clarified from the outset (see Moran *et al.*, 2004). Traditionally, biological control programmes have not been considered successful unless there has been a reduction in population density of existing stands of the weed. Using this yardstick, the introduction of a single species of seed-reducing biological control agent, such as *M. servulus*, is unlikely to achieve the goal. With the large seed loads that are produced annually by *A. cyclops*, even high levels of seed destruction mean that sufficient seed will still escape to find safe sites and then the self-thinning principle (Silverton and Lovette Doust, 1993), applies to limit the population density of plants. When agents that affect plant fecundity are being used, success may be better gauged if effective control means that spread away from infestations is reduced, and that the long-term management of the weed becomes easier, and cheaper, through an integrated

biological and mechanical control operation (Moran *et al.*, 2004), then it is possible for a seed-reducing agent like *M. servulus* to have a positive impact.

Bearing in mind that there were constraints placed on the type of agents that could be used, the selection of *M. servulus* under the circumstances was well founded for several reasons: i) the use of a seed-reducing agent was totally acceptable to those people wanting to use *A. cyclops* as a source of firewood; ii) the programme in South Africa using seed-feeding weevils against *Hakea sericea*, had shown promising results and set a precedent for the use of such agents (Kluge and Neser, 1991); iii) seed attacking agents were accepted because of their tendency to be very host-specific (Janzen, 1971) as well as being easy to collect and adapted well to South African conditions and, vi) seed-reducing agents should theoretically reduce the rate of spread and thus contribute to the control of the weed (Harper, 1977; Macdonald and Jarman, 1984; Harley, 1985; Neser and Kluge, 1986; Moody and Mack, 1988). Whether the reduction in seed production of *A. cyclops* by *M. servulus* will ultimately translate into a reduction in population density remains, at this stage, an unknown factor.

This study was undertaken to determine levels of seed destruction in canopy-held pods of *A. cyclops*, and to look at the dispersal abilities of *M. servulus*, and its interactions with indigenous alydid seed-feeders, as well as the effects of alydid and bird feeding on subsequent germination of the seed. The results showed that *M. servulus* has the ability to achieve and maintain very high levels of seed destruction annually, unlike the native alydid seed-feeders, which are too sporadic and generally infrequent. In addition, unless there are constraints which exclude them from some regions, *M. servulus* will in time spread throughout the range of *A. cyclops* in South Africa, either unassisted, or via re-distribution efforts. The full potential of *M. servulus* has not yet been realized since populations of the weevil are still increasing and spreading, however the prognosis for the future is promising.

The role of *M. servulus* in the control of *A. cyclops* is seen as being part of a larger, integrated, management programme, which includes other biological control agents combined with mechanical clearing. During the latter part of this study a second biological control agent, the flower-galler, *Dasineura dielsi* (Diptera: Cecidomyiidae), was released against *A. cyclops* (Adair, 2004). This agent also suppresses reproductive

growth by ovipositing on the flowers, which then form galls rather than seed pods, and has rapidly become widespread on *A. cyclops* in South Africa (Adair, 2004). The release of the second agent has provided much scope for future research, and it is hoped, as with *Trichilogaster acaciaelongifoliae* and *M. ventralis* on *A. longifolia* (Dennill and Donnelly, 1991; Donnelly and Hoffmann, 2004), that the two agents will be much more effective in combination than alone.

Despite the recent introduction of *D. dielsi* into the system, this study on the impacts of *M. servulus*, has laid the groundwork for an investigation into the effects of such seed-reduction on future management prospects for *A. cyclops*. A long-term study has recently been initiated to look at seedling recruitment and survival of *A. cyclops* growing in different situations both with and without the influence of biological control. Through such a study the impact of *M. servulus* on *A. cyclops* populations, should become apparent.

The control of invasive trees and shrubs is probably one of the biggest tasks managers and landowners in South Africa are faced with in the future, and without the integration of biological control in the system, there is little hope of sustained success. In addition, with the introduction of the "Working for Water" initiative in South Africa, and the mechanical clearing of large areas of invasive plants, it is critical that biological control is recognized, incorporated, and maintained as a crucial component of the programme, and through this the benefits will be reaped long into the future.

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